EXHIBIT 101

EPA

FINAL DRAFT:

TECHNICAL SUPPORT DOCUMENT FOR A PROTOCOL TO ASSESS ASBESTOS-RELATED RISK

Prepared for:

Office of Solid Waste and Emergency Response
U.S. Environmental Protection Agency
Washington, DC 20460

NOTICE

This document provides guidance to EPA staff. It also provides guidance to the public and to the regulated community on how EPA intends to exercise its discretion in implementing the National Contingency Plan. The guidance is designed to implement national policy on these issues. The document does not, however, substitute for EPA's statutes or regulations, nor is it a regulation itself. Thus, it cannot impose legally-binding requirements on EPA, States, or the regulated community, and may not apply to a particular situation based upon the circumstances. EPA may change this guidance in the future, as appropriate.

U.S. Environmental Protection Agency

Authors

D. Wayne Berman
Aeolus, Inc.
751 Taft St.
Albany, CA 94706

Kenny S. Crump
Environ Corporation
602 East Georgia Ave.
Ruston, LA 71270

Contributors/Reviewers

Affiliation: McGill University
Name: Bruce Case, Associate Professor
Location: 462 Argyle Avenue Westmount, Quebec H3Y 3B4 Canada
Phone: 514-398-7192 #00521
Fax: 514-398-7446
Email: bruce.case@mcgill.ca

Affiliation: Pathology & Physiology Research Branch National Institute for Occupational Safety & Health
Name: Vincent Castranova, Chief
Location: 1095 Willowdale Road (L 2015) Morgantown, WV 26505
Phone: 304-285-6056
Fax: 304-285-5938
Email: vic1@cdc.gov

Affiliation: Department of Medicine National Jewish Medical Research Center
Name: James Crapo, Chairman
Location: 1400 Jackson Street Denver, CO 80206
Phone: 303-398-1436
Fax: 303-270-2243
Email: crapoj@njc.org

Affiliation: Medical University of South Carolina
Name: David Hoel, Professor
Location: 36 South Battery Charleston, SC 29401
Phone: 843-723-1155
Fax: 843-723-7405
Email: whitepoint@aol.com

Affiliation: New York University School of Medicine
Name: Morton Lippmann, Professor
Location: 57 Old Forge Road Tuxedo, NY 10987
Phone: 845-731-3558
Fax: 845-351-5472
Email: lippmann@env.med.nyu.edu

Affiliation: Toxicology & Human Health Risk Analysis
Name: Roger McClellan, Advisor
Location: 13701 Quaking Aspen Place, NE Albuquerque, NM 87111
Phone: 505-296-7083
Fax: 505-296-9573
Email: roger.o.mcclellan@att.net

Affiliation: Price Associates, Inc.
Name: Bertram Price
Location: 1 North Broadway - #406 White Plains, NY 10601
Phone: 914-686-7975
Fax: 914-686-7977
Email: bprice@priceassociatesinc.com

Affiliation: California Environmental Protection Agency
Name: Claire Sherman, Biostatistician
Location: 1515 Clay Street, 16th Floor Oakland, CA 94612
Phone: 510-622-3214
Fax: 510-622-3211
Email: csherman@oehha.ca.gov

Affiliation: Risk Evaluation Branch National Institute for Occupational Safety & Health
Name: Leslie Thomas Stayner, Chief
Location: Robert Taft Laboratories, C15 4676 Columbia Parkway Cincinnati, OH 45226
Phone: 513-533-8365
Fax: 513-533-8224
Email: lts2@cdc.gov

Affiliation: Rollins School of Public Health, Emory University
Name: Kyle Steenland, Professor
Location: 1518 Clifton Road Atlanta, GA 30322
Phone: 404-712-8277
Email: nsteenl@sph.emory.edu

Affiliation: Exponent, Inc.
Name: Mary Jane Teta, Principal Epidemiologist
Location: 234 Old Woodbury Road Southbury, CT 06488
Phone: 203-262-6441
Fax: 203-262-6443
Email: jteta@exponent.com

ACKNOWLEDGMENTS

We wish to acknowledge the guidance and support for this project provided by Aparna Koppikar, Richard Troast, Chris Weis, and Paul Peronard (all of U.S. EPA).

We wish to gratefully acknowledge the assistance of Nick de Klerk, FDK Liddell, J. Corbett McDonald, Terri Schnoor, and John Dement for graciously providing the raw epidemiology data from several key studies, without which we could not have completed our analysis.

We also wish to thank John Addison, John Dement, Agnes Kane, Bruce Case, Michel Camus, and Vanessa Vu for their input and assistance.

Finally, we wish to acknowledge Eric Hack and Tammie Covington for their assistance with calculations and data management and Mark Follansbee and Joanne White for their assistance with technical editing and managing production of the document.

ACRONYMS

AM	alveolar macrophages
AP	alkaline phophatase
ATSDR	American Toxic Substances Disease Registry
BAL	bronchio-alveolar lavage
BrdU	bromodeoxyuridine
CalEPA	California Environmental Protection Agency
CFE	colony-forming efficiency
CHO	Chinese hamster ovary
EDXA	energy dispersive X-ray analysis
EGFR	Epithelial Growth Factor Receptor
ERK	EGFR-regulated kinase
ESR	electron spin residence
FBP's	fibrin breakdown products
HAF	human amniotic fluid
HBE	human bronchiolar epithelial
HNE	human neutrophil elastase
HTE	hamster tracheal epithelial
IPF	idiopathic pulmonary fibrosis
IRIS	Integrated Risk Information System
KGF	kertinocyte growth factor
LDH	lipid dehydrogenase
LPS	lipopolysaccharide
MAPK	mitogen activated protein kinase
MI	midget impinger
MLE	maximum likelihood estimate
MMVF's	man-made vitreous fibers
MnSOD	manganese-containing superoxide
mpcf	millions of particles per cubic foot
mppcf	millions of dust particles per cubic foot
MSHA	Mine Safety and Health Administration
NAC	N-acetylcysteine
NHBE	normal human bronchioepithelial
NHIS	National Health Interview Survey
NIOSH	National Institute for Occupational Safety and Health
OSHA	Occupational Safety and Health Agency
PARS	poly-ADP-ribosyl transferase
PCM	phase contrast microscopy
PE	pulmonary epithelial
PMN	polymorphonucleocyte
RBC's	red blood cells
RCF	refractory ceramic fiber
RNS	reactive nitrogen species
ROS	reactive oxygen species
RPM	rat pleural mesothelial
RR	relative risk
SAED	selected area electron diffraction
SEM	scanning electron microscopy
SHE	Syrian Hamster Embryo
SMG	small mucous granule
SMRs	standardized mortality ratios
THE	tracheal epithelial cells
TEM	transmission electron microscopy
TGF-β	transforming growth factor beta
TNF-α	tumor necrosis factor alpha
uPA	urokinase-type plasminogen activator
uPAR	urokinase-type plasminogen activator
U.S. EPA	U.S. Environmental Protection Agency
VCAM-1	vascular cell adhesion molecule

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1.0 EXECUTIVE SUMMARY

The purpose of this report is to provide a foundation for completing a state-of-the-art-protocol to assess potential human-health risks associated with exposure to asbestos. Such a protocol is intended specifically for use in performing risk assessments at Superfund sites, although it may be applicable to a broad range of situations.

The current report is a revision to a version originally submitted on September 4, 2001 (Berman and Crump 2001), which was the subject of a peer-review consultation held in San Francisco on February 25–26, 2003. In general, the expert panel endorsed the overall approach to risk assessment proposed in this report, although they highlighted areas where controversies persist.

The current report incorporates the changes recommended by the peer review consultation panel to correct minor problems with internal consistency and the overall transparency of the discussion that are needed to improve readability. Although some of the research and analyses recommended by the peer consultation panel are not complete, it is anticipated that the current document can be distributed for broader review and comment. Thus, the recommended approach to risk assessment can be considered for use in the interim, while the additional research and analyses recommended by the expert panel are completed. At that point, a final revision of this document will be developed and it is expected to serve as a component of a broader effort by the U.S. Environmental Protection Agency (U.S. EPA) to revise the Agency's current approach for assessing asbestos-related risks.

The approach currently employed at the U.S. EPA to evaluate asbestos-related risks (IRIS 1988) is based primarily on a document completed in 1986 (U.S. EPA 1986) and has not been changed substantially in the past 15 years, despite substantial improvements in asbestos measurement techniques and in the understanding of the manner in which asbestos exposure contributes to disease. Therefore, this document provides an overview and evaluation of the more recent studies and presents proposed modifications to the protocol for assessing asbestos-related risks that can be justified based on the more recent work.

As reported in several recent technical meetings and reinforced by information gleaned from the literature, the following were identified as issues that need to be addressed to develop a protocol for evaluating asbestos-related risk:

- whether the exposure-response models currently in use by the U.S. EPA for describing the incidence of asbestos-related diseases adequately reflect the time- and exposure-dependence for the development of these diseases;
- whether different potencies need to be assigned to the different asbestos mineral types to adequately predict risk for the disease endpoints of interest;
- to the extent that different asbestos mineral types are assigned distinct potencies, whether the relative *in vivo* durability of different asbestos mineral types determines their relative potency;

- whether the set of minerals included in the current definition of asbestos adequately covers the range of minerals that potentially contribute to asbestos-related diseases;

- whether the analytical techniques and methods currently used for determining asbestos concentrations adequately capture the biologically relevant characteristics of asbestos (particularly with regard to the sizes of the structures counted using the various analytical methods) so that they can be used to support risk assessment; and

- whether reasonable confidence can be placed in the cross-study extrapolation of exposure-response relationships that are required to assess asbestos-related risks in new environments of interest.

These outstanding issues (and other related considerations) are addressed in this document to provide a foundation for proposing a new approach for assessing asbestos-related risks. Although the objective of this evaluation was to identify the single best procedure, when current knowledge is inadequate for distinguishing among alternatives, options are presented along with a discussion of their relative advantages and limitations. In a few cases, limited and focused additional research studies are recommended, which may enhance the current state of knowledge sufficiently to resolve one or more of the important, remaining issues.

Background

Inhalation of asbestos dusts has been linked to several adverse health effects including primarily asbestosis, lung cancer, and mesothelioma (U.S. EPA 1986). Asbestosis, a chronic, degenerative lung disease, has been documented among asbestos workers from a wide variety of industries. Although asbestosis cases have been observed at some locations of current interest to the U.S. EPA, the disease is generally expected to be associated only with the higher levels of exposure commonly found in workplace settings and is not expected to contribute substantially to potential risks associated with environmental asbestos exposure. Therefore, asbestosis is only considered in this document to the extent required to address its putative association with lung cancer. Overall, the majority of evidence indicates that lung cancer and mesothelioma are the most important risks associated with exposure to low levels of asbestos.

The Asbestos Literature

A variety of human, animal, and tissue studies have provided insight into the nature of the relationship between asbestos exposure and disease. Ideally, human epidemiology studies are employed to determine the quantitative exposure-response relationships and the attendant risk coefficients for asbestos exposure. Exposure-response coefficients have been estimated for asbestos from approximately 20 epidemiology studies for which adequate exposure-response data exist. Such coefficients vary widely, however, and the observed variation has not been reconciled. Among the objectives of this study is to evaluate and account for the sources of uncertainty that contribute to the variation among the exposure-response coefficients derived from the literature so that these estimates can be reasonably interpreted and recommendations for their use in risk assessment developed.

Animal and tissue studies indicate that asbestos potency is a complex function of several characteristics of asbestos dusts including fiber size and fiber type (i.e., fiber mineralogy). Moreover, the influence of fiber size is a complex function of both diameter and length. Therefore, whenever the goal is to compare across samples with differing characteristics, it is not sufficient to report asbestos concentrations simply as a function of mass (or any other single measure), which is in stark contrast to the treatment of chemical toxins. It has generally been difficult to distinguish among the effects of fiber size and type in many studies because such effects are confounded and the materials studied have not been adequately characterized.

The Epidemiology Studies

The existing epidemiology studies provide the most appropriate data from which to determine the relationship between asbestos exposure and response in humans. As previously indicated, however, due to a variety of methodological limitations, the ability to compare and contrast results across studies needs to be evaluated to determine the confidence with which results from existing epidemiology studies may be extrapolated to new environments where risk needs to be assessed. This requires both that the uncertainties contributed by such methodological limitations and that several ancillary issues be addressed.

Briefly, the major kinds of limitations that potentially contribute to uncertainty in the available epidemiology studies include:

- limitations in air measurements and other data available for characterizing historical exposures;
- limitations in the manner that the character of exposure (i.e., the mineralogical types of fibers and the range and distribution of fiber dimensions) was delineated;
- limitations in the accuracy of mortality determinations or incompleteness in the extent of tracing of cohort members;
- limitations in the adequacy of the match between cohort subjects and the selected control population; and
- inadequate characterization of confounding factors, such as smoking histories for individual workers.

The existing asbestos epidemiology database consists of approximately 150 studies of which approximately 35 contain exposure data sufficient to derive quantitative exposure/response relationships. A detailed evaluation of 20 of the most recent of these studies, which includes the most recent follow-up for all of the cohorts evaluated in the 35 studies, was completed. The following conclusions result from this evaluation:

(1) To study the characteristics of asbestos that relate to risk, it is necessary to combine results (i.e., in a meta analysis) from studies of environments having asbestos dusts of differing characteristics. More robust conclusions regarding risk

can be drawn from an analysis of the set of epidemiology studies taken as a whole than results derived from individual studies.

(2) By adjusting for fiber size and fiber type, the existing database of studies can be reconciled adequately to reasonably support risk assessment.

(3) The U.S. EPA models for lung cancer and mesothelioma both appear to track the time-dependence of disease at long times following cessation of exposure. However, the relationship between exposure concentration and response may not be adequately described by the current models for either disease. There is some evidence that these relationships are supra-linear.

(4) Whereas the U.S. EPA model for lung cancer assumes a multiplicative relationship between smoking and asbestos, the current evidence suggests that the relationship is less than multiplicative, but possibly more complex than additive. However, even if the smoking-asbestos interaction is not multiplicative as predicted by the U.S. EPA model, exposure-response coefficients estimated from the model are still likely to relate to risk approximately proportionally and, consequently, may be used to determine an exposure index that reconciles asbestos potencies in different environments. However, adjustments to the coefficients may be required in order to use them to estimate absolute lung cancer risk for differing amounts of smoking. This issue needs to be investigated further in the next draft of this document.

(5) The optimal exposure index that best reconciles the published literature assigns equal potency to fibers longer than 10 μm and thinner than 0.4 μm and assigns no potency to fibers of other dimensions.

(6) The optimal exposure index also assigns different exposure-response coefficients for chrysotile and amphibole both for lung cancer and mesothelioma. For lung cancer the best estimate of the coefficient (potency) for chrysotile is 0.27 times that for amphibole, although the possibility that chrysotile and amphibole are equally potent cannot be ruled out. For mesothelioma the best estimate of the coefficient (potency) for chrysotile is only 0.0013 times that for amphibole and the possibility that pure chrysotile is non-potent for causing mesothelioma cannot be ruled out by the epidemiology data.

(7) Using the approach recommended in the U.S. EPA (1986) update, the lung cancer exposure-response coefficients (K_L values) estimated from 15 studies vary by a factor of 72 and these values are mutually inconsistent (based on non-overlap of uncertainty intervals). Using the approach based on the optimal exposure index that is recommended herein, the overall variation in K_L values across these studies is reduced to a factor of 50.

(8) Using the approach recommended in the U.S. EPA (1986) update, the mesothelioma exposure-response coefficients (K_M values) estimated from 10 studies vary by a factor of 1,089 and these values are likewise mutually

inconsistent. Using the approach based on the optimal exposure index that is recommended herein, the overall variation in K_M values across these studies is reduced to a factor of 30.

(9) The exposure index and exposure-response coefficients embodied in the risk assessment approach developed in this report are more consistent with the literature than the current U.S. EPA approach. In particular, the current approach appears highly likely to seriously underestimate risk from amphiboles, while possibly overstating risk from chrysotile. Furthermore, most of the remaining uncertainties regarding the new proposed approach also apply to the current approach. Consequently, it is recommended that the proposed approach begin to be applied in assessment of asbestos risk on an interim basis, while further work, as recommended below, is conducted to further refine the approach.

(10) The residual inconsistency in both the lung cancer and mesothelioma potency values is primarily driven by those calculated from Quebec chrysotile miners and from South Carolina chrysotile textile workers. The difference in the lung cancer potency estimated between these studies has long been the subject of much attention. A detailed evaluation of the studies addressing this issue, the results of our analysis of the overall epidemiology literature, and implications from the broader literature, indicate that the most likely cause of the difference between these studies is the relative distribution of fiber sizes in the two environments. It is therefore likely that the variation between these studies can be further reduced by developing improved characterizations of the dusts that were present in each of these environments (relying on either archived samples, or newly generated samples using technologies similar to those used originally).

Recommendations for Risk Assessment

Although gaps in knowledge remain, a review of the literature addressing the health-related effects of asbestos (and related materials) provides a generally consistent picture of the relationship between asbestos exposure and the induction of disease (lung cancer and mesothelioma). Therefore, the general characteristics of asbestos exposure that drive the induction of cancer can be inferred from the existing studies and were applied to define appropriate procedures for evaluating asbestos-related risk.

Optimum values for exposure-response coefficients for lung cancer and mesothelioma were derived in this analysis and can be combined with appropriately defined exposure estimates as inputs for the U.S. EPA lung cancer and mesothelioma models (respectively) to assess risk. Although these values are optimized within the constraints of the current analysis and reduce the apparent variation across published studies substantially, the need to manage and minimize risk when developing a general approach for assessing risk, is also recognized. Thus, to reduce the chance of under-estimating risks, a conservative set of potency estimates were also developed and are also presented. To assess risk, depending on the specific application, either the best-estimate risk coefficients or the conservative estimates can be incorporated into procedures described herein for assessing asbestos-related risks.

Tables are also provided that present estimates of the additional risk of death from lung cancer, from mesothelioma, and from the two diseases combined that are attributable to lifetime, continuous exposure at an asbestos concentration of 0.0001 f/cm^3 (for fibrous structures longer than 10 μm and thinner than 0.4 μm) as determined using TEM recommended methods. The risk estimates in these tables can be combined with appropriately determined estimates of exposure to develop estimates of risk in environments of interest.

Recommendations for Limited, Further Study

The two major objectives identified for further study are:

(1) to evaluate a broader range of exposure-response models in fitting the observed relationship between asbestos exposure and lung cancer or mesothelioma, respectively. For lung cancer models, this would also include an attempt to better account for the interaction between asbestos exposure and smoking; and

(2) to develop the supporting data needed to define adjustments for exposure-response coefficients that will allow them to be used with an exposure index that more closely captures the criteria that determine biological activity. Among other things, this work should focus on obtaining data that would permit more complete reconciliation of the exposure-response coefficients derived for Quebec miners and South Carolina textile workers.

2.0 INTRODUCTION

The purpose of this report is to provide a foundation for completing a state-of-the-art-protocol to assess potential human-health risks associated with exposure to asbestos. Such a protocol is intended specifically for use in performing risk assessments at Superfund sites, although it may be applicable to a broad range of situations.

The current report is a revision to a version originally submitted on September 4, 2001 (Berman and Crump 2001), which (among other things) includes both an extensive review of the general literature and a detailed analysis of the existing epidemiology studies. These are reproduced in the current report in Chapter 6 and Chapter 7/Appendix A (respectively).

The September 4, 2001 version was also the subject of a peer-review consultation held in San Francisco on February 25–26, 2003. The comments of the expert panel convened to conduct the peer review are included in this report as Appendix B.

In general, the expert panel endorsed the overall approach to risk assessment proposed in this report, although they highlighted areas where controversies persist. They also suggested additional research and analyses to attempt to resolve some of the outstanding controversies and to refine several of the details of the approach. In addition, they offered recommendations for modifications to improve the overall transparency and readability of the earlier version of this report.

The current report incorporates the changes recommended by the peer review consultation panel to correct minor problems with internal consistency and the overall transparency of the discussion that are needed to improve readability. Although some of the research and analyses recommended by the peer consultation panel are not complete, it is anticipated that the current document can be distributed for broader review and comment. Thus, the recommended approach to risk assessment can be considered for use in the interim, while the additional research and analyses recommended by the expert panel are completed. At that point, a final revision of this document will be developed and it is expected to serve as a component of a broader effort by the U.S. Environmental Protection Agency (U.S. EPA) to revise the Agency's current approach for assessing asbestos-related risks.

The approach currently employed by U.S. EPA to evaluate asbestos-related risks (IRIS, 1988) is based primarily on a document completed in 1986 (U.S. EPA 1986) and has not changed in the past 15 years, despite substantial improvements in asbestos measurement techniques and in the understanding of the manner in which asbestos exposure contributes to disease. Therefore, among other things, this document provides an overview and evaluation of more recent studies and presents proposed modifications to the current approach for assessing asbestos-related risks that can be justified based on the more recent work.

In May 2001, the U.S. EPA along with the California Environmental Protection Agency (CalEPA), the National Institute for Occupational Safety and Health (NIOSH), the American Toxic Substances Disease Registry (ATSDR), and the Mine Safety and Health Administration (MSHA) hosted an international conference on asbestos in Oakland, California that was attended

by leading international experts on asbestos. The state of knowledge concerning such issues as the nature of asbestos, the measurement of asbestos, and the relationship between asbestos exposure and the induction of disease was reviewed during this conference. Particular emphasis was placed on identifying important knowledge gaps in these areas.

By coupling the outstanding issues identified at the Oakland meeting with additional information gleaned from the literature, the following set of issues was identified as risk-specific issues of current interest:

- whether the exposure-response models currently in use by the U.S. EPA for describing the incidence of asbestos-related diseases adequately reflect the time- and exposure-dependence for the development of these diseases;
- whether different potencies need to be assigned to the different asbestos mineral types to adequately predict risk for the disease end points of interest;
- to the extent that different asbestos mineral types are assigned distinct potencies, whether the relative *in vivo* durability of different asbestos mineral types determines their relative potency;
- whether the set of minerals included in the current definition of asbestos adequately covers the range of minerals that potentially contribute to asbestos-related diseases;
- whether the analytical techniques and methods currently used for determining asbestos concentrations adequately capture the biologically relevant characteristics of asbestos (particularly with regard to the sizes of the structures counted using the various analytical methods) so that they can be used to support risk assessment; and
- whether reasonable confidence can be placed in the cross-study extrapolation of exposure-response relationships that are required to assess asbestos-related risks in new environments of interest.

These outstanding issues (and other related considerations) are addressed in this document to provide a foundation for proposing a new approach for assessing asbestos-related risks. Compared to the current U.S. EPA approach, it is shown that the new approach better predicts risks among the environments in which asbestos-related risks have been previously evaluated (i.e., the published epidemiology studies) so that the new approach can be used to predict risks in unstudied environments of interest with greater confidence than predictions based on the current approach. Moreover, completing the additional research and analysis recommended by the expert panel (Appendix B) should facilitate further refinement while providing additional opportunities to better evaluate and validate the proposed approach.

The remainder of this document is divided into 6 chapters:

- Chapter 3 presents an overview of the general considerations that need to be addressed to assess asbestos-related risks (including considerations associated with the manner in which asbestos exposures are characterized, the manner in which risk is modeled from existing data, and the manner that risk models are then applied to estimate risk in new environments). The nature of the diseases commonly attributed to asbestos exposure are also briefly described;

- Chapter 4 presents a background discussion that addresses the definition of asbestos, the mineralogy of asbestos, the morphology of asbestos-containing dusts to which people are typically exposed, the capabilities and limitations of analytical techniques and methods used to determine airborne asbestos concentrations, and the structure and function of the human lung;

- Chapter 5 provides a description of the kinds of literature studies that are commonly used to support development of a protocol to assess risk, with particular emphasis on identifying their relative strengths and weaknesses;

- Chapter 6 presents a review of the literature with particular emphasis on studies published since the Health Effects Assessment Update (U.S. EPA 1986). Combined with a description of supporting analyses, the review is focused on reconciling apparently conflicting studies and hypotheses (when possible) and identifying the best candidate procedures for assessing asbestos-related risks. To reconcile studies, the strengths and weaknesses common to various types of studies are explicitly considered;

- Chapter 7 presents a reevaluation of the published epidemiology studies that, among other things, is designed to address and (when possible) resolve the outstanding issues of current interest; and

- Chapter 8 presents a proposed, new approach for assessing asbestos-related risks.

Regarding Chapter 8, although the objective of this document was to identify the single best procedure, when current knowledge is inadequate for distinguishing among alternatives, options are presented along with a discussion of their relative advantages and limitations. A few limited and focused additional research studies are recommended, which have the potential to enhance the current state of knowledge sufficiently to resolve one or more of the important, remaining issues. These recommended studies parallel those identified by the peer review panel (Appendix B).

This report is part of a series of documents developed as part of a multi-task project to develop a set of mutually consistent methods for determining asbestos concentrations in a manner useful for assessing risk and a companion protocol for conducting such risk assessments. A method for the determination of asbestos in air (Chatfield and Berman 1990) and a companion technical background document (Berman and Chatfield 1990) were published by the U.S. EPA in 1990. The air method has since been superceded (improved) by the ISO Method (ISO 1995). A

method for the determination of asbestos in soils and bulk materials (Berman and Kolk 1997) was also published by the U.S. EPA and the draft of an improved version was also recently completed (Berman and Kolk 2000). The recommendations in this document should serve as the basis for development of the companion risk-assessment protocol.

3.0 OVERVIEW

Inhalation of asbestos dusts has been linked to several adverse health effects including primarily asbestosis, lung cancer, and mesothelioma (U.S. EPA 1986). The kinds of lung cancers linked to asbestos exposure are similar to those induced by smoking and a greater-than-additive effect has been observed from combined exposure (see, for example, Liddell and Armstrong 2002). Mesothelioma is a rare cancer of the membranes that line the pleural cavity (containing the heart and lungs) and the peritoneal cavity (i.e., the gut). Although there is some evidence of a low background incidence of spontaneous mesotheliomas, this cancer has been associated almost exclusively with exposure to asbestos and certain other fibrous substances (HEI-AR, 1991).

Asbestosis, a chronic, degenerative lung disease, has been documented among asbestos workers from a wide variety of industries. Although asbestosis cases have been observed at some locations of current interest to the U.S. EPA, the disease is generally expected to be associated only with the higher levels of exposure commonly found in workplace settings and is not expected to contribute substantially to risks potentially associated with environmental asbestos exposure. Therefore, asbestosis is only considered in this document to the extent required to address its putative association with lung cancer. Overall, the majority of evidence indicates that lung cancer and mesothelioma are the most important risks associated with exposure to low levels of asbestos.

> The primary route of exposure of concern in association with asbestos is inhalation. There is little evidence that ingestion of asbestos induces disease (see, for example, IRIS 1988; U.S. EPA 1986). Therefore, this study is focused on inhalation hazards, and other routes of exposure are not addressed.

Gastrointestinal cancers and cancers of other organs (e.g., larynx, kidney, and ovaries) have also been linked with asbestos exposures (by inhalation) in some studies. However, such associations are not as compelling as those for lung cancer and mesothelioma and the potential risks from asbestos exposures associated with these other cancers are much lower (U.S. EPA 1986). Consequently, by addressing the more substantial asbestos-related risks associated with lung cancer and mesothelioma, the much more moderate risks potentially associated with cancers at other sites are also addressed by default. Therefore, this document is focused on the risks associated with lung cancer and mesothelioma.

A variety of human, animal, and tissue studies have provided insight into the nature of the relationship between asbestos exposure and disease. Ideally, human epidemiology studies are employed to determine the quantitative exposure/response relationships and the attendant risk coefficients for asbestos exposure. Risk coefficients have been estimated for asbestos from approximately 20 epidemiology studies for which adequate exposure-response data exist. However, such coefficients vary widely (for lung cancer, coefficients vary by more than a factor of 70 and, for mesothelioma, they vary by more than a factor of 1,000) and this variation has not been reconciled. Among the objectives of this study, one is to evaluate and account for the sources of uncertainty that contribute to the variation among the risk coefficients derived from the literature so that these estimates can be reasonably interpreted and recommendations for their use in risk assessment developed.

Animal and tissue studies indicate that asbestos potency is a complex function of several characteristics of asbestos dusts including fiber size and fiber type (i.e., fiber mineralogy). Moreover, the influence of fiber size is a complex function of both diameter and length. Therefore, whenever the goal is to compare across samples with differing characteristics, it is not sufficient to report asbestos concentrations simply as a function of mass (or any other single parameter), which is in stark contrast to the treatment of chemical toxins. It has generally been difficult to distinguish among the effects of fiber size and type in many studies because such effects are confounded and the materials studied have not been adequately characterized.

The influence of different characteristics of asbestos dusts upon risk cannot be adequately evaluated in the existing epidemiological studies because the analytical techniques used to monitor asbestos exposure in these studies are not capable of resolving all of the characteristics of asbestos dusts that other types of studies indicate are important. Moreover, the exposure indices (the range of structure sizes and shapes used to characterize an asbestos dust) that are employed in the existing epidemiology studies may not correspond with the characteristics of asbestos that best relate to biological activity. This hinders the ability to reconcile risk (exposure-response) coefficients derived from different studies. It also limits the confidence with which risk coefficients derived from the existing epidemiology studies can be applied to assess risks from asbestos exposure in other environments. Such limitations are explored in this report, along with potential remedies.

Based on the current approach for evaluating asbestos-related cancer risk (U.S. EPA 1986), risk is estimated as the product of a risk coefficient and a mathematical function that depends on the level of exposure, the duration of exposure, and time. The risk coefficient for lung cancer is generally denoted as, "K_L" and the one for mesothelioma as "K_M". A detailed description of both the current lung cancer and mesothelioma models is provided in Chapter 7. The models differ depending on whether lung cancer or mesothelioma is being considered.

For lung cancer, the model estimates *relative* risk, which means that the increase in lung cancer incidence that is attributable to asbestos exposure is proportional to the background lung cancer incidence in the exposed population. The background cancer incidence is the rate of lung cancer that would be expected to occur in the population in the absence of asbestos exposure. In other words, background lung cancer incidence is the lung cancer rate for the exposed population that is attributable to all causes other than asbestos.

Among the implications of the lung cancer model is that the combined effects of asbestos exposure and smoking is multiplicative and, until recently, the majority of studies have suggested such a multiplicative relationship (see, for example, Hammond et al. 1979). However, newer studies (for example, Liddell and Armstrong 2002) suggest a complex relationship that is closer to additive than multiplicative. Such considerations are addressed further in Chapter 7.

The current EPA model for mesothelioma is an *absolute* risk model. This means that the increase in mesothelioma risk attributable to asbestos is independent of the background rate of mesothelioma, which is negligible in the general population.

Ideally, the risk coefficients derived from the existing epidemiology studies can be combined with measurements from other exposure settings to estimate lung cancer and mesothelioma risks in these other exposure settings. However, such risk estimates are only valid if both of the following conditions are met:

(1) asbestos is measured in the exposure setting of interest in the identical manner in which it was measured in the study from which the corresponding risk coefficients are derived; and

(2) such measurements reflect the characteristics of asbestos exposures that determine risk.

A growing body of evidence indicates that the way in which asbestos concentrations were measured in the existing epidemiology studies do not reflect the characteristics of asbestos exposure that determine risk. Therefore, measuring asbestos concentrations in the same way in exposure settings of interest may not be sufficient to assure the validity of risk estimates derived using the published risk coefficients (and the corresponding models). This is because the second of the above-listed conditions would not be satisfied.

Considerations necessary to compare risk coefficients derived in different exposure settings (or to apply a coefficient to predict risk in a setting different from the one in which the coefficient was derived) have been elucidated clearly in a mathematical model (Chesson et al. 1990). The consequences of the model indicate that adjusting the existing risk coefficients so that they reflect asbestos characteristics that determine biological activity requires knowledge of the fiber size distributions of the dusts studied in the *original* epidemiology studies. To the extent they exist, such data may be used to normalize each of the published risk coefficients so that they relate to a common exposure index reflecting asbestos characteristics that determine biological activity.

Among the goals of this evaluation is to explore the possibility of defining an improved exposure index (that better reflects biological activity) and to use this index to reconcile the epidemiology data (see Chapter 7). We also evaluated improved ways of simultaneously accounting for the effects of both fiber size and type.

Unfortunately, some of the issues that need to be resolved to support development of a protocol for assessing asbestos-related risks cannot be entirely resolved with existing data. Therefore, in later chapters (i.e., Chapters 6, 7, and 8), we have attempted to identify such issues, to assess their relative importance, and, when deemed appropriate, to propose limited and focused research projects designed to provide the data required to reduce the impacts of such knowledge gaps.

4.0 BACKGROUND

Asbestos is a term used to describe the fibrous habit of a family of hydrated metal silicate minerals. The most widely accepted definition of asbestos includes the fibrous habits of six of these minerals (IARC 1977). The most common type of asbestos is chrysotile, which is the fibrous habit of the mineral serpentine, a magnesium silicate. The other five asbestos minerals are all amphiboles (i.e., all partially hydrolyzed, mixed-metal silicates). These are: fibrous reibeckite (crocidolite), fibrous grunerite (amosite), anthophyllite asbestos, tremolite asbestos, and actinolite asbestos.

All six of the minerals whose fibrous habits are termed asbestos occur most commonly in non-fibrous, massive habits. While unique names have been assigned to the asbestiform varieties of three of the six minerals (noted parenthetically above) to distinguish them from their massive forms, such nomenclature has not been developed for anthophyllite, tremolite, or actinolite. Therefore, when discussing these latter three minerals, it is important to specify whether a massive habit of the mineral or the fibrous (asbestiform) habit is intended.

Although other minerals may also occur in a fibrous habit, they are not generally included in the definition of asbestos either because they do not exhibit properties typically ascribed to asbestos (e.g., high tensile strength, the ability to be woven, heat stability, and resistence to attack by acid or alkali) or because they do not occur in sufficient quantities to be exploited commercially.

The first four of the six asbestos minerals listed above have been exploited commercially (IARC 1977). Of these, chrysotile alone accounts for more than 90% of the asbestos found in commercial products.

Importantly, it is neither clear whether the term asbestos maps reasonably onto the range of fibrous minerals that can contribute to asbestos-like health effects nor whether individual structures of the requisite mineralogy must formally be asbestiform to contribute to such health effects.

Regarding whether the term asbestos is a useful discriminator for health effects, it is well established that erionite (a fibrous zeolite not related to asbestos) is a potent inducer of mesothelioma (Baris et al. 1987), which is one of the two primary asbestos-induced cancers (see Chapter 3). It is therefore possible that the fibrous habits of at least some other minerals not formally included in the current definition of asbestos may contribute to the induction of asbestos-related diseases. Therefore, an efficient procedure is needed for separating potentially hazardous materials from those that are most likely benign.

There are two issues related to the question of whether fibers must formally be asbestiform to contribute to health effects. The first involves the relationship between fiber structure and disease induction and the second involves measurement. Although the evidence is overwhelming that the size and shape of a fiber affects the degree to which it contributes to the induction of disease (this is addressed in detail in Chapter 6), it does not appear that sizes inducing biological activity are well distinguished by criteria that define the asbestiform habit. Therefore, depending on the definition employed for the fibers (or fibrous structures) that are

counted during an analysis, it may or may not be necessary to distinguish formally between asbestiform and non-asbestiform structures for the concentrations derived from such a count to adequately reflect biological activity.

The dimensions of an asbestiform fiber are determined by the manner in which the fiber grows (Addison 2001). In contrast, the massive forms of various minerals, when cleaved, also form elongated particles (termed "cleavage fragments") and, depending on the definition employed for fibrous structures during an analysis, such cleavage fragments may or may not be included along with asbestiform fibers in a count (see Section 4.3). Although it is clear from the manner in which they are each formed that the surface properties of asbestiform fibers and cleavage fragments are likely to be very different (for example, the latter will have many "unsatisified" chemical bonds), the degree to which such differences affect the toxic potency for comparable sized structures is not currently known.

Although it is beyond the scope of this document to present a detailed treatise on asbestos mineralogy, the morphology of asbestos dusts, or the nature and limitations of analytical techniques and methods used to determine asbestos concentrations, a brief overview of these topics is presented in the following sections both to identify issues that need to be addressed as part of the development of an appropriate protocol for assessing asbestos risks and to provide the background required to facilitate evaluation of the relevant issues. In that regard, a section on lung physiology and function is also provided.

4.1 THE MINERALOGY OF ASBESTOS

As previously indicated, the six asbestos minerals can be divided into two general classes. Chrysotile is the fibrous habit of the mineral serpentine (Hodgson 1965). The smallest fibrils of chrysotile occur as rolled sheets or hollow tubules of this magnesium silicate mineral. The larger fibers of chrysotile form as tightly packed bundles of the unit fibrils.

Chrysotile fibrils typically range from 20 nm to approximately 300 or 400 nm (0.02 to 0.3 or 0.4 μm) in diameter. Although slightly thicker fibrils may occasionally occur, at some point the curvature induced by the mismatch between the magnesium and silicon layers of the fibril becomes thermodynamically unstable, so that production of thicker fibrils is prohibited (Addison 2001).

Chrysotile bundles are held together primarily by Van der Waals forces and will readily disaggregate in aqueous solutions containing wetting agents (e.g., soap). They will also readily disaggregate in lung surfactant (Addision, 2001).

The general chemical composition of serpentine is reported as $Mg_3(Si_2O_5)(OH)_4$ (Hodgson 1965). However, the exact composition in any particular sample may vary somewhat from the general composition. For example, aluminum may occasionally replace silicon and iron, nickel, manganese, zinc or cobalt may occasionally replace magnesium in the crystal lattice of chrysotile (serpentine).

The five other common varieties of asbestos are all fibrous forms of amphibole minerals (Hodgson 1965). These are ferro-magnesium silicates of the general composition:

$$A_{2-3}B_5(Si,Al)_8O_{22}(OH)_2$$

where:

A = Mg, Fe, Ca, Na, or K; and
B = Mg, Fe, or Al.

Some of these elements may also be partially substituted by Mn, Cr, Li, Pb, Ti, or Zn.

The fibrous habits of the amphibole minerals tend to occur as extended chains of silica tetrahedra that are interconnected by bands of cations (Hodgson 1965). Each unit cell typically contains eight silica tetrahedra and the resulting fibers tend to be rhomboid in cross-section. Amphibole fibers are generally thicker than chrysotile fibrils and may typically range from approximately 100 nm to 700 or 800 nm in diameter (Addison 2001). Substantially thicker fibers have also been observed.

4.2 MORPHOLOGY OF ASBESTOS DUSTS

Structures comprising the fibrous habits of the asbestos minerals come in a variety of shapes and sizes. Not only do single, isolated fibers vary in length and thickness, but such fibers may be found combined with other fibers to form bundles (aggregates of closely packed fibers arranged in parallel) or clusters (aggregates of randomly oriented fibers) or combined with equant particles to form matrices (asbestos fibers embedded in non-asbestos materials). Consequently, dusts (even of one mineral variety) are complex mixtures of structures. For precise definitions of the types of fibrous structures typically found in asbestos dusts, see ISO (1995).

Detailed descriptions of the characteristics of dusts typically encountered at environmental and occupational asbestos sites have been reported in the literature and the following summary is based on a previously published review (Berman and Chatfield 1990). Typically, the major components of the dust observed in most environments are non-fibrous, isometric particles. Fibrous structures consistently represent only a minor fraction of total dust. Asbestos structures represent a subset of the fibrous structures.

The magnitude of the fraction of total dust represented by fibers and the fraction of fibers composed of asbestos minerals vary from site to site. However, the fraction of asbestos in total dusts has been quantified only in a very limited number of occupational and environmental settings (see, for example, Cherrie et al. 1987 or Lynch et al. 1970).

The gross features of structure size distributions appear to be similar among asbestos dusts characterized to date (Berman and Chatfield 1990). The major asbestos fraction of all such dusts are small fibrous structures less than 5 μm in length. Length distributions generally exhibit a mode (maximum) between 0.8 and 1.5 μm with larger fibers occurring with decreasing frequency. Fibrous structures longer than 5 μm constitute no more than approximately 25% of total asbestos structures in any particular dust and generally constitute less than 10%.
In some environments, the diameters of asbestos fibers exhibit a narrow distribution that is

largely independent of length. In other environments, diameters appear to exhibit a narrow distribution about a mean for each specific length. In the latter case, both the mean and the spread of the diameter distribution increases as the length of the structures increase. The increase in diameter with length appears to be more pronounced for chrysotile than for the amphiboles, presumably due to an increase in the fraction of chrysotile bundles contributing to the overall distribution as length increases.

Only a few studies have been published that indicate the number of complex structures in asbestos size distributions. The limited data available indicate that complex structures may constitute a substantial fraction (up to one third) of total structures, at least for chrysotile dusts (see, for example, Sebastien et al. 1984). Similar results were also obtained during a re-analysis of dusts generated from the asbestos samples evaluated in the animal inhalation studies conducted by Davis et al. (Berman et al., in preparation). This is the same re-analysis used to support a study to identify asbestos characteristics that promote biological activity (Berman et al. 1995), which is discussed further in Section 6.4.3.

Historically, fibrous structures have been arbitrarily defined as structures exhibiting aspect ratios (the ratio of length to width) greater than 3:1 to distinguish them from isometric particles (Walton 1982). However, alternate definitions for fibers have also been proposed, which are believed to better relate to biological activity (see, for example, Berman et al. 1995 or Wylie et al. 1993). The degree to which fibers are combined within complex structures in a particular dust may also affect the biological activity of the dust (Berman et al. 1995). Therefore, proper characterization of asbestos exposure requires that the relative contributions from each of many components of exposure be simultaneously considered. Factors that need to be addressed include the distribution of structure sizes, shapes, and mineralogy in addition to the absolute concentration of structures. Such considerations are addressed further in Chapter 6. Thus, unlike the majority of other chemicals frequently monitored at hazardous wastes sites, asbestos exposures cannot be adequately characterized by a single concentration variable.

4.3 CAPABILITIES OF ANALYTICAL TECHNIQUES USED TO MONITOR ASBESTOS

Due to a complex history, a range of analytical techniques and methods have been employed to measure asbestos in the various studies conducted over time (Walton 1982). Use of these various methods has affected the comparability of results across the relevant asbestos studies (Berman and Chatfield 1990). Therefore, the relative capabilities and limitations of the most important methods used to measure asbestos are summarized here. Later sections of this report incorporate attempts to reconcile effects that are attributable to the limitations of the different methods employed in the various studies evaluated.

Analytical techniques used to measure airborne asbestos concentrations vary greatly in their ability to fully characterize asbestos exposure. The capabilities and limitations of four analytical techniques (midget impinger [MI], phase contrast microscopy [PCM], scanning electron microscopy [SEM], and transmission electron microscopy [TEM]) are described here. A general comparison of the relative capabilities and limitations of the analytical techniques introduced above is presented in Table 4-1.

Table 4-1. Capabilities and Limitations of Analytical Techniques Used for Asbestos Measurements[a]

Parameter	Midget Impinger	Phase Contrast Microscopy	Scanning Electron Microscopy	Transmission Electron Microscopy
Range of Magnification	100	400	2,000–10,000	5,000–20,000
Particles Counted	All	Fibrous Structures[b]	Fibrous Structures[b]	Fibrous Structures[b,c]
Minimum Diameter (size) Visible	1 μm	0.3 μm	0.1 μm	< 0.01 μm
Resolve Internal Structure	No	No	Maybe	Yes
Distinguish Mineralogy[d]	No	No	Yes	Yes

[a]The capabilities and limitations in this table are based primarily on the physical constraints of the indicated instrumentation. Differences attributable to the associated procedures and practices of methods in common use over the last 25 years are highlighted in Table 4-2.
[b]Fibrous structures are defined here as particles exhibiting aspect ratios (the ratio of length to width) greater than 3 (see Walton 1982).
[c]TEM counts frequently resolve individual fibrous structures within larger, complex structures. Based on internal structure, several different counting rules have been developed for handling complex structures. See the discussion of methods presented below.
[d]Most SEM and TEM instruments are equipped with the capability to record selected area electron diffraction (SAED) spectra and perform energy dispersive X-ray analysis (EDXA), which are used to distinguish the mineralogy of structures observed.

MI and PCM are the two analytical techniques used to derive exposure estimates in the majority of epidemiology studies from which the existing risk factors are derived. SEM is an analytical technique that has been employed in several key animal studies. TEM provokes interest because it is the only analytical technique that is potentially capable of distinguishing all of the characteristics of asbestos that potentially affect biological activity.

Although PCM was (and still is) widely used to characterize occupational exposures, its inability to distinguish between asbestos and non-asbestos and its lack of sensitivity limits its usefulness in environmental settings (Berman and Chatfield 1990). In fact, PCM analyses and TEM analyses showed no correlation among measurements collected during the cleanup of the 1991 Oakland Hills fire (Berman, unpublished data). Such lack of correlation is expected to be observed generally whenever measurements are collected at sites where asbestos concentrations are low enough that a substantial fraction of the structures counted by PCM are not asbestos. Consequently, TEM is the technique that has been recommended for use at Superfund sites (Berman and Kolk 1997; Chatfield and Berman 1990).

Importantly, the physical limitations of the various analytical techniques is only part of the problem. To provide reproducible results that can be compared meaningfully to other analyses in other studies, one must also consider the choice of procedures (methods) that address everything from sample collection and preparation to rules for counting and quantifying asbestos structures.

Table 4-2. Comparison of Applicable Methods For Measuring Asbestos in Air

	NIOSH 7400[a]	NIOSH 7402[b]	YAMATE[c]	AHERA[d]	ISO[e]
Analytical Technique	PCM	TEM	TEM	TEM	TEM
Preparation Methodology	Direct (no transfer)	Direct	Direct (Indirect Optional)	Direct	Direct (Indirect Optional)
Magnification	450x	10,000x	20,000x	15,000x - 20,000x	20,000x (total structures) 10,000x (structures longer than 5 µm)
Dimensions Counted					
Length (L):	L > 5 µm	L > 1 µm	L > 0.06 µm	L > 0.5 µm	L > 0.5 µm (total structures) L > 5 µm (long structures)
Width (W):	W < 0.25 µm	3.0 > W > 0.04 µm	W > 0.02 µm	W > 0.02 µm	W < 3.0 µm (resolution)
Aspect Ratio (AR):	AR > 3	AR > 3	AR > 3	AR > 5	AR > 3
Sensitivity:					
s/cm³				0.005	Adjustable
s/mm²				70	10 for total structures 100 for long structures
Mineralogy Determined	No	Yes	Yes, except matrix particles	Yes, except matrix particles	Yes
Maximum Number Counted	100 structures	100 structures	100 structures	50 structures	100 total structures 100 long structures

Table 4-2. Comparison of Applicable Methods For Measuring Asbestos in Air *(continued)*

	NIOSH 7400[a]	NIOSH 7402[b]	YAMATE	AHERA[d]	ISO
Maximum Area Scanned	100 fields	100 grid openings	10 grid openings	Blanks: 10 openings Samples: 10 openings (assuming defined sensitivity is achieved with the collected air volumes).	Adjustable
Statistically Balanced Counting[g]		No	No	No	Yes
Counting Rules					
Structures	Count all structures exhibiting L > 5 µm, W < 3.0 µm, and AR > 3.	Count all structures exhibiting L > 1 µm, W < 3.0 µm, and AR > 3. Note PCME[h] fraction within count.	Count all structures exhibiting an AR > 5.	Count all structures with L > 0.5 µm exhibiting an AR > 5. Record individual fibers within all groupings with fewer than 3 intersections. Count structures with L > 5 µm separately (PCME[g]).	Count all structures with L > 0.5 µm or counting components with L > 0.5 µm that also exhibit an AR > 5. Separately identifiable components of parent structures that satisfy the dimensional criteria are also separately enumerated. Conduct a similar count to that indicated above for structures with L > 5 µm.

Table 4-2. Comparison of Applicable Methods For Measuring Asbestos in Air *(continued)*

	NIOSH 7400[a]	NIOSH 7402[b]	YAMATE	AHERA[d]	ISO
Bundles	Bundles meeting overall dimensional criteria generally counted as single fibers unless up to 10 individual fiber ends can be distinguished within the bundle, representing individual fibers	Bundles meeting overall dimensional criteria generally counted as single fibers.	Bundles meeting overall dimensional criteria generally counted as single entities and noted as bundles on the count sheet.	Bundles of 3 or more fibers that meet the overall dimensional criteria are counted as single entities and noted as bundles on the count sheet.	Count parent bundles with L ≥ 10 µm containing at least one countable fiber that exhibits an AR ≥ 3. Qualifying bundles that are components of other parent structures are also separately enumerated. For counts of structures with L ≥ 5 µm, include only bundles longer than 5 µm.
Clusters	Within a cluster, count up to 10 individual fiber ends from up to 5 fibers that meet the overall dimensional criteria. Otherwise, count as a single entity.	Within a cluster, count up to 3 individual fibers that meet the overall dimensional criteria. Otherwise, clusters that contain more than 3 fibers that meet the overall dimensional criteria are counted as single clusters.	Within a cluster, count up to 5 individual fibers that meet the overall dimensional criteria. Otherwise, clusters that contain more than 5 fibers that meet the overall dimensional criteria are counted as single clusters.	A collection of fibers with more than 2 intersections where at least one individual projection meets the overall dimensional criteria is counted as a single cluster.	Discretely dispersed and compact clusters. Count all clusters containing at least one countable fiber or bundle satisfying appropriate dimensional criteria. Separately enumerate up to five component structures satisfying appropriate dimensional criteria.

Table 4-2. Comparison of Applicable Methods For Measuring Asbestos in Air *(continued)*

	NIOSH 7400	NIOSH 7402[b]	YAMATE[c]	AHERA[d]	ISO[e]
Matrices	Count up to 5 fibers emanating from a cluster (matrix). Each individual fiber must meet the dimensional criteria.	Count individually identifiable fibers within a matrix. Fibers must individually meet the dimensional criteria.	Count as a single matrix all matrices with at least one protruding or embedded fiber that meets the dimensional criteria.	Count as a single matrix, all matrices with at least one protruding fiber such that the protruding section meets the dimensional criteria.	Distinguish dispersed and compact matrices. Count all matrices containing at least one component fiber or bundle satisfying appropriate dimensional criteria. Separately enumerate up to 10 component structures satisfying appropriate dimensional criteria.

[a]National Institute for Occupational Safety and Health (1985). *Method for Determination of Asbestos in Air Using Positive Phase Contrast Microscopy.* NIOSH Method 7400. NIOSH, Cincinnati, Ohio, U.S.A.

[b]National Institute for Occupational Safety and Health (1986). *Method for Determination of Asbestos in Air Using Transmission Electron Microscopy.* NIOSH Method 7402. NIOSH, Cincinnati, Ohio, U.S.A.

[c]Yamate, G., Agarwal, S.C., and Gibbons, R.D. (1984). *Methodology for the Measurement of Airborne Asbestos by Electron Microscopy.* U.S. EPA Report No. 68-02-3266. U.S. Environmental Protection Agency, Washington, D.C., U.S.A.

[d]U.S. Environmental Protection Agency (1987). *Asbestos Hazard Emergency Response Act: Asbestos-Containing Materials in Schools.* Final Rule and Notice (Appendix A: AHERA Method). Federal Register, 40 CFR 763, Vol. 52, No. 2, pp. 41826-41903, October.

[e]Chatfield, E.J. (1995). *Ambient Air: Determination of Asbestos Fibres, Direct Transfer Transmission Electron Microscopy Procedure.* Submitted to the International Standards Organization: ISO/TC 10312.

[f]Note that the ISO Method is a successor to the Interim Superfund Method: Chatfield, E.J. and Berman, D.W. (1990). *Superfund Method for the Determination of Asbestos in Ambient Air. Part I: Method Interim Version.*. Prepared for the U.S. Environmental Protection Agency, Office of Emergency and Remedial Response, Washington, D.C. EPA/540-2-90/005a. May.

[g]Statistically balanced counting is a procedure incorporated into some asbestos methods (e.g. the Superfund Methods and the ISO Methods) in which long structures (typically longer than 5 μm) are counted separately during a lower magnification scan than used to count total structures (which are predominantly short). This procedure assures that the relatively rare longer structures are enumerated with comparable precision to that of the shorter structures.

[h]PCME stands for phase contrast microscope equivalent and indicates the fraction of structures observed by transmission electron micrscopy that would also be visible by phase contrast microscopy.

Multiple methods have been published for use in conjunction with several of the analytical techniques mentioned above (particularly TEM). Such methods differ in the procedures incorporated for sample preparation and for the manner in which asbestos structures are counted. The sample preparation requirements, conditions of analysis, and structure counting rules for several of the most commonly employed methods are presented in Table 4-2 to illustrate how the choice of method can result in substantially different measurements (even on duplicate or split samples).

The second column of Table 4-2 describes the specifications of the PCM method currently mandated by the Occupational Safety and Health Agency (OSHA) for characterizing asbestos exposure in occupational settings. Although this method is in common use today, several alternate methods for counting fibrous structures by PCM have also been used historically. Therefore, PCM measurements reported in earlier studies (including the available epidemiology studies) may not be comparable to PCM results collected today.

The last four columns of Table 4-2 describe TEM methods that are in current use. Comparison across these methods indicates:

- the shortest lengths included in counts using these methods vary between 0.06 and 1 μm. Given that structures shorter than 1 μm represent a substantial fraction of total asbestos structures in almost any environment (Section 4.2), this difference alone contributes substantially to variation in measurement results across methods;

- the definitions and procedures for counting complex structures (i.e., bundles, clusters, and matrices) vary substantially across methods, which further contribute to variation in measurement results. For example, the ISO Method requires that component fibers of clusters and matrices be counted separately, if they can be readily distinguished. In contrast, clusters are counted as single structures under the AHERA Method; and

- although all of the methods listed incorporate sample preparation by a direct transfer process (in which the fibers are counted in their original position on the filter), several of the methods have also been paired with an optional indirect transfer process (which involves ashing the original air filter, mixing the residue in water, sonicating, and re-suspending the fibers on a new filter). Measurements derived from split samples that are prepared, respectively, by direct and indirect transfer, can vary by factors as large as several 100 (Berman and Chatfield 1990). Typically, counts of asbestos structures on samples prepared by an indirect transfer procedure are greater than those derived from directly prepared samples by factors of between 5 and 50.

Given the combined effects from the physical limitations of the various techniques employed to analyze for asbestos and the varying attributes of the methods developed to guide use of these techniques, the relative capabilities and limitations of asbestos measurements derived, respectively, from paired methods and techniques in common use can be summarized as follows:

- all four techniques are particle counting techniques;
- neither MI nor PCM are capable of distinguishing asbestos from non-asbestos (i.e., they are incapable of determining structure mineralogy);
- counting rules used in conjunction with MI do not distinguish isometric particles from fibers;
- counting rules used in conjunction with PCM limits counting to fibrous structures longer than 5 μm with aspect ratios greater than 3:1;
- the range of visibility associated with PCM limits counting to fibers thicker than approximately 0.3 μm;
- under conditions typically employed for asbestos analysis, the range of visibility associated with SEM limits counting to fibers thicker than approximately 0.1 μm, which is only marginally better than PCM;
- SEM is capable of distinguishing asbestos structures from non-asbestos structures;
- TEM is capable of resolving asbestos structures over their entire size range (down to thicknesses of 0.01 μm);
- TEM is capable of distinguishing the internal components of complex asbestos structures; and
- TEM is capable of distinguishing asbestos structures from non-asbestos structures.

More detailed treatments of the similarities and differences between asbestos techniques and methods can also be found in the literature (see, for example, Berman and Chatfield 1990).

Due to the differences indicated, measurements from a particular environment (even from duplicate samples) that are derived using different analytical techniques and methods can vary substantially and are not comparable. In fact, results can differ by two or three orders of magnitude (Berman and Chatfield 1990). More importantly, because the relative distributions of structure sizes and shapes vary from environment to environment, measurements derived using different analytical techniques and methods do not even remain proportional from one environment to the next. Therefore, the results from multiple asbestos studies can only be meaningfully compared if the effects that are attributable to use of differing analytical techniques and methods can be quantified and reconciled. Few of the existing studies, however, document analytical procedures in sufficient detail to reconstruct exactly what was done.

4.4 THE STRUCTURE AND FUNCTION OF THE HUMAN LUNG

4.4.1 Lung Structure

The lungs are the organs of the body in which gas exchange occurs to replenish the supply of oxygen and eliminate carbon dioxide. To reach the gas exchange regions of the lung, inhaled air (and any associated toxins) must first traverse the proximal conducting (non-respiratory) airways of the body and the lung including the nose (or mouth), pharynx, larynx, trachea, and the various branching bronchi of the lungs down to the smallest (non-respiratory) bronchioles. Air then enters the distal (respiratory) portion of the lung, where gas exchange occurs.

In humans, the respiratory portion of the lungs are composed of the respiratory or aveolarized bronchioles, the alveolar ducts, and the alveoli (or alveolar sacs). There are approximately $3x10^8$ alveoli in human lungs (about 20 per alveolar duct) with a cumulative volume of $3.9x10^3$ cm^3 (Yeh and Harkema 1993). This represents approximately 65% of the total volume capacity of human lungs at full inspiration.

Yeh and Harkema (1993) also report that the ratio of lung volume to body weight is approximately constant across a broad range of mammals (from a shrew, 0.007 kg to a horse, 500 kg). Human lung volumes average a little more than 5 L.

Each human alveolus has a diameter of approximately 0.03 cm (300 μm) and a length of 0.025 cm (Yeh and Harkema 1993). The typical path length from the trachea to an alveolus is approximately 25 cm. The bronchi leading to each alveolus have branched an average of 16 times from the trachea (with a range of 9 to 22 branches). Importantly, the mean path length, the number of branches between trachea and alveolus, and the detailed architecture (branching pattern) of the respiratory region of the lung vary across mammalian species. For example, rats and mice lack respiratory bronchioles while macaque monkeys exhibit similar numbers of respiratory bronchiole generations as humans (Nikula et al. 1997). Furthermore, branching in humans tends to be symmetric (each daughter branch being approximately the same size and the angle of branching for each is similar but not quite equal) while rodents tend to exhibit monopodal branching in which smaller branches tend to come off at angles from a main trunk (Lippmann and Schlesinger 1984).

The gas exchange regions of the lung are contained within the lung parenchyma, which constitute approximately 82% of the total volume of the lungs (Gehr et al. 1993). Importantly, the lung parenchyma is not a "portion" of the lung; it fills virtually the entire volume of the organ traditionally visualized as the "whole" lung. Embedded within the parenchyma are the larger conducting airways of the lungs and the conducting blood vessels that transport blood to and from the capillaries that are associated with each alveolus. In the human lung, approximately 213 ml of blood are distributed over 143 m^2 of gas-exchange (alveolar) surface area (about the size of a tennis court). The gas-exchange surface area of lungs scale linearly with body weight over most mammalian species. The slope of the "reduced" line (where the Y-Axis is the ratio of the surface area to the surface area in a reference species and the X-Axis is the ratio of the body mass to the body mass of the same reference species) is 0.95. Figure 4-1 is a photomicrograph showing two views of a portion of lung parenchyma in the vicinity of a terminal bronchiole and an avleolar duct, which are labeled. The circular spaces in the left

portion of the figure and the cavities in the right portion of the figure are alveoli. Note the thinness of the walls (septa) separating alveoli.

Figure 4-1. The Structure of Lung Parenchyma Showing Alveoli and Alveolar Ducts (Source: St. George et al. 1993)

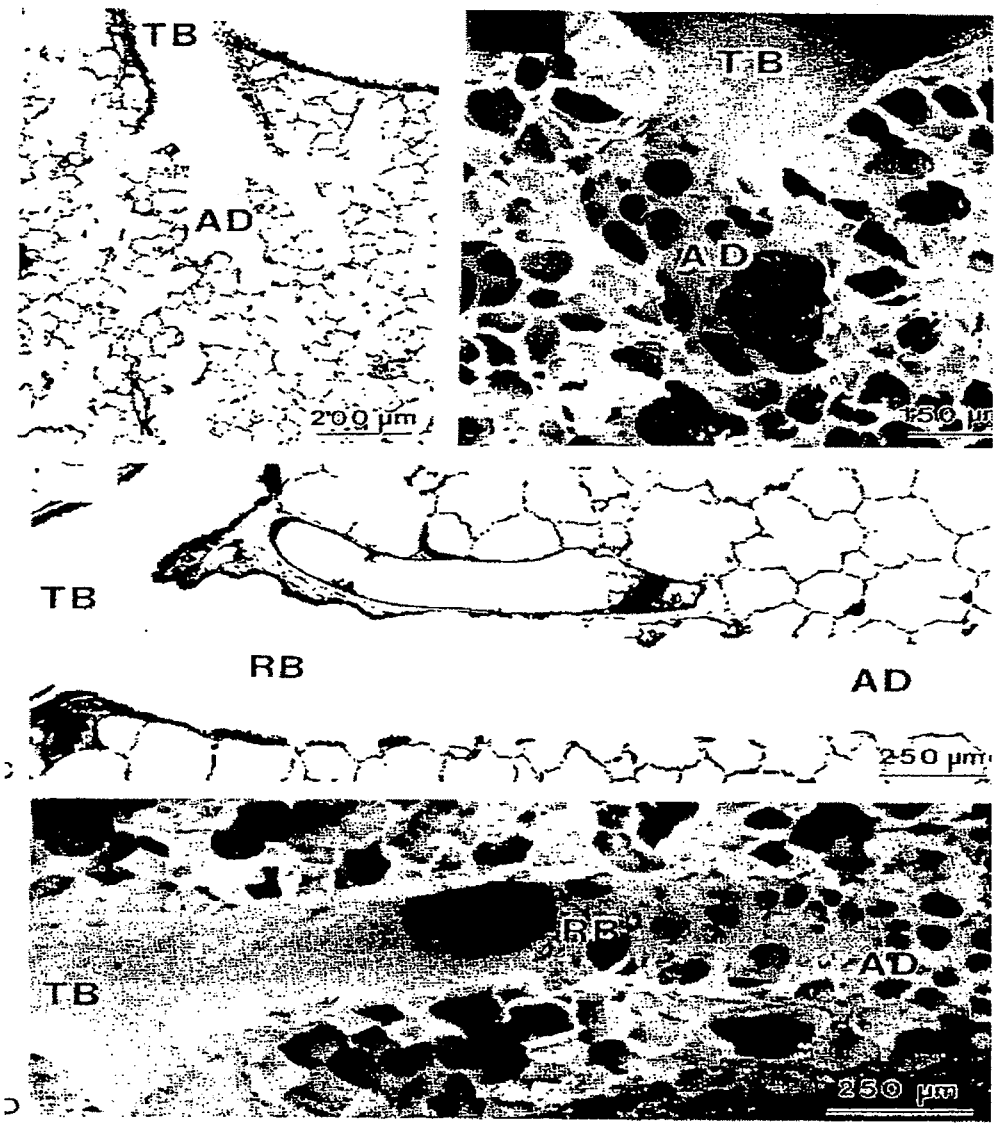


FIG. 10. LM and SEM comparison of the centriacinar region with two different organizations and B: The short or poorly developed respiratory bronchiole of the rat; C and D, the w alveolarized respiratory bronchiole of the cat. Terminal bronchiole (TB), respiratory bronchi (RB), and alveolar duct (AD). For details see ref. 106.

Confidential: Need Permission to Reproduce this Figure

Alveoli are separated from each other by alveolar septa that average only a few micrometers in thickness (Gehr et al. 1993). Gas-exchange capillaries run within these septa and the air-blood barrier, which averages only 0.62 μm in thickness, is composed of three layers: the alveolar epithelium, the interstitium, and endothelium. The epithelium is described in detail below. The interstitium is primarily composed of a collagenous, extracellular matrix, which constitute about two-thirds of the interstitial volume. There is also a collection of fibroblasts, macrophages, and other cells interspersed within the matrix (Miller et al. 1993). The cells of the interstitium constitute about one third of its volume. The endothelial layer is composed of the smooth muscle cells and connective tissue that constitute the walls of vascular capillaries. The amount of connective tissue in the septa between alveoli also varies between animal species; small rodents have less and primates more (Nikula et al. 1997).

Figures 4-2 and 4-3 show, respectively, a typical alveolar septum and a closeup of one portion of such a septum. In Figure 4-2, one can see that the septa themselves are thin and are filled almost entirely with capillaries. Figure 4-3 shows that the epithelial lining of an alveolus is no more than 1 μm thick, that the underlying interstitium is no thicker, and that the endothelium of the adjacent capillary is similarly thin. These three layers constitute the major tissues of the air-blood barrier (the rest of the barrier includes the limited quantity of blood plasma between the endothelial wall of a capillary and a red blood cell and the outer membrane of the red blood cell itself).

Figure 4-2. The Structure of the Inter-Alveolar Septa (Source: Gehr et al. 1993)

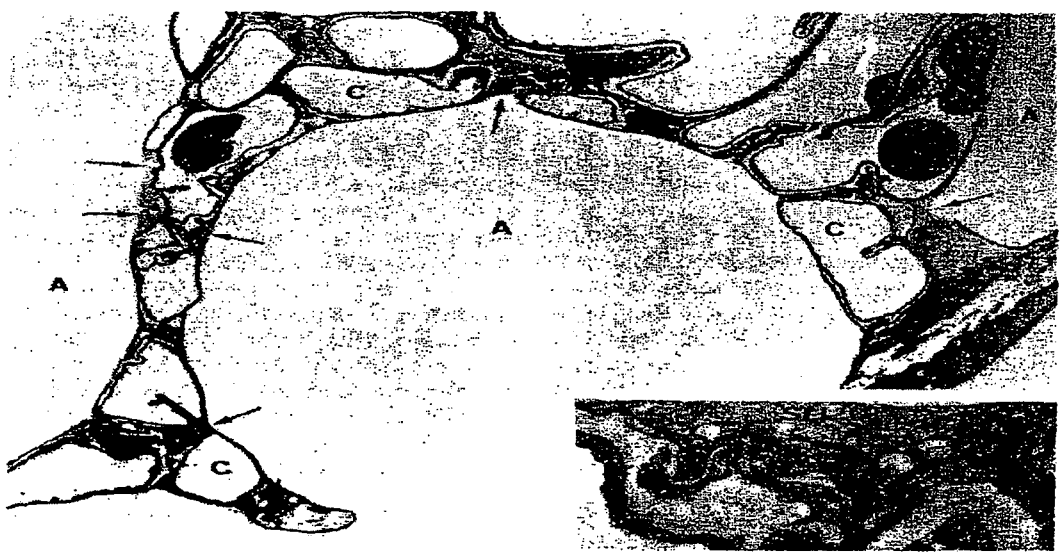


FIG. 10. Transmission electron micrograph of an alveolus from a dog lung fixed by intravascular perfusion. The lung was inflated with air at a pressure of 5 cm of water on the deflation limb of the last of three hysteresis cycles (approximately 60% TLC). It shows interalveolar septa that are folded at this degree of inflation. The surface lining layer (SLL) smoothes out every depression of the alveolar surface (*arrows*). A, alveoli; C, capillary. ×2,100. *Inset*: High power view of an epithelial depression filled with a fluid surface lining layer (SLL). ×14,600.

Confidential: Need Permission to Reproduce this Figure

Figure 4-3. The Detailed Structure of an Alveolar Wall that is Part of an Inter-Alveolar Septum (Source: Gehr et al. 1993)

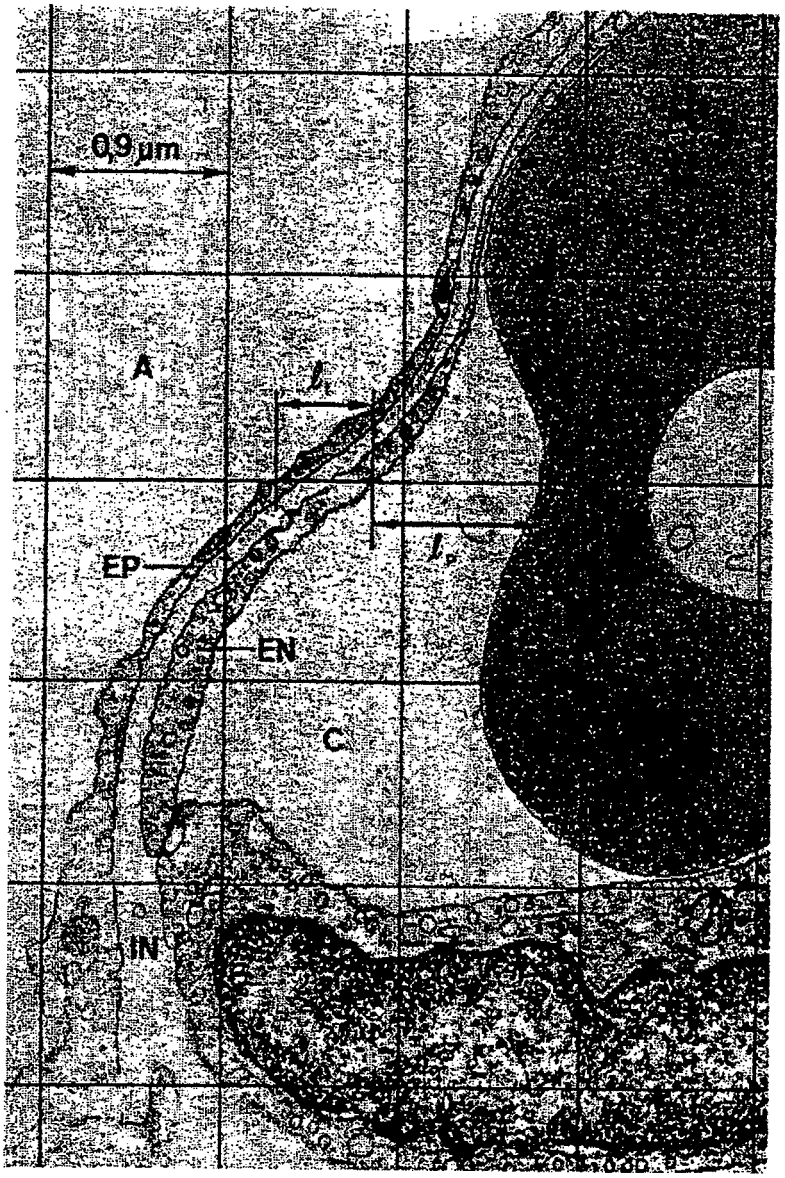


FIG. 19. Fraction of square lattice test grid superimposed on alveolar capillary to measure intercept lengths in tissue (l_t) and plasma (l_p) for the calculation of the harmonic mean barrier thickness. Note the erythrocyte (EC) in capillary (C) and that the barrier separating blood from alveolar air (A) is made of the three layers epithelium (EP), endothelium (EN), and interstitium (IN). ×29,000. (From ref. 80.)

Confidential: Need Permission to Reproduce this Figure

Gehr et al. (1993) also report that interalveolar septa constitute approximately 14% of the volume of the gas-exchange region of the lung (i.e., the lung parenchyma). Of this tissue mass, approximately 20% is endothelium, 55% is interstitium, and the rest is composed of cells associated with the alveolar epithelium. The remainder of the gas-exchange region is air space. Gehr et al. (1993) also report that the interalveolar septa fold to accommodate changes in lung volume during respiration, although the major contribution to lung volume changes (over the range of normal inspiration) appears to be the collapsing (folding) of alveolar ducts (Mercer and Crapo 1993).

According to Gehr et al. (1993), Type I epithelial cells, which constitute approximately 95% of the surface area of alveolar epithelium, are flat and platy (squamous), and average less than 1 μm in thickness (except where cell nuclei exist, which are approximately 7.5 μm in diameter and protrude into the alveolar space). Type II epithelial cells, which are cuboidal, constitute no more than 5% of the epithelial surface. Despite the small fraction of surface that they occupy, Type II cells serve to maintain the integrity of the overall epithelial lining so that, for example, they limit the tissue's permeability and control/prevent transport of macromolecules from the interstitium to the alveolar space, or the reverse (Leikauf and Driscoll 1993). Type II cells also secrete lung surfactant. The basement membrane of alveolar epithelium is a collagenous structure.

In contrast, the epithelial cells lining the trachea and bronchi (including the respiratory bronchioles) are ciliated and columnar and averages between 10 and 15 μm in thickness (based on photomicrographs presented in St. George et al. 1993). Tracheobronchial epithelium reportedly contains at least 8 distinct cell types (St. George et al. 1993): ciliated epithelium, basal cells (small flat cells situated along the basal lamina and not reaching the luminal surface), mucous goblet cells, serous cells, nonciliated bronchiolar (clara) cells, small mucous granule (SMG) cells, brush cells, and neuroendocrine cells. The relative abundance of the various cells varies across mammalian species as well as across the various airway generations (branches) and even the opposing sides of specific airways. The number of cells per length of basal lamina also varies across mammalian species.

4.4.2 The Structure of the Mesothelium

The mesothelium is a double layered membrane and each layer is a single-cell thick. The two layers of the mesothelium are separated by a space (e.g., the pleural space), which contains extracellular fluid and free macrophages (Kane and McDonald 1993). The pleural space is drained at fixed locations by lymphatic ducts. Each layer of the mesothelium overlies a collagenous basement membrane containing dispersed spindle cells. Depending on the location of the mesothelium, the basement membrane may overlie the skeletal muscle of the diaphragm or the rib cage (in the case of the parietal pleura, which is the outer layer). For the inner layer or visceral pleura, the basement membrane overlies visceral organs (including the lungs) within the rib cage. Healthy mesothelium is quiescent, meaning that cells are not actively dividing.

The relative size and thickness of mesothelial tissue varies across mammalian species (Nikula et al. 1997). For example, rats have relatively thin pleura with limited lymphatic ducts. In contrast, nonhuman primates have thicker pleura with greater lymphatic drainage than rats and humans have even thicker pleura and relatively abundant lymphatic ducts.

4.4.3 Cytology

Alveolar Epithelium. In the respiratory region of the lung, Type II epithelial cells are progenitor cells for Type I epithelium (Leikauf and Driscoll 1993). Type I epithelial cells are not proliferation competent (Nehls et al. 1997). After injury to Type I cells, Type II cells proliferate and reestablish the continuous epithelial surface. Type II cells also secrete surfactant. It appears that the identity and location of the progenitor cells for Type II epithelial cells are not currently known.

Injury or alteration of Type II cell function are associated with several diseases included idiopathic pulmonary fibrosis (Leikauf and Driscoll 1993). Also, crystalline silica and other toxic agents have been shown to directly modify Type II cellular activity. For example, crystalline silica (at sub-cytotoxic levels, <100 μg/ml) stimulates Type II proliferation in tissue culture. In contrast, neither titanium dioxide nor aluminum coated silica induce proliferation at corresponding concentrations.

In culture, Type II epithelial cells transform slowly into Type I cells and thus have limited population doubling capacity (Leikauf and Driscoll 1993). Rarely can the number of Type II cells expand past 3–10 passages (20–30 doublings). During this time, cells terminally differentiate, develop cross-linked envelopes, and appear squamous, enlarged, and multinucleated. The process is noted to be accelerated by the presence of transforming growth factor beta (TGF-β).

Macrophages. Alveolar macrophages (the largest population of macrophages in the lung) are mobile, avidly phagocytic, present antigens, and release cytokines that trigger various other immune responses (Leikauf and Driscoll 1993). They also initiate inflammatory responses and other repair mechanisms that are designed to restore tissue homeostasis.

The next largest population of macrophages in the lung are the interstitial macrophages (Leikauf and Driscoll 1993). These are localized in the peribronchial and perivascular spaces, the interstitial spaces of the lung parenchyma, the lymphatic channels, and the visceral pleura. The various populations of macrophages in the lung express different surface proteins, show different proliferative capacity, and show differences in metabolism.

The sizes of alveolar macrophages varies substantially across mammalian species (Krombach et al. 1997). Krombach and co-workers provide a table summary of the relative sizes across several species of interest:

Animal	Mean diameter (μm)	Mean Volume (μm^3)
Rats	13.1±0.2	1166±42
Syrian Golden Hamsters	13.6±0.4	1328±123
Cyanomolgus Monkeys	15.3±0.5	
Healthy Humans	21.2±0.3	4990±174

Note: as indicated later (Section 6.2), the relative size of various macrophages has direct implications regarding the dependence of clearance mechanisms on fiber size.

Mesothelium. Importantly, mesothelial cells are proliferation competent and may be their own progenitor cells (Kane and McDonald 1993). It is also possible, however, that as yet-to-be-identified progenitor cells are located along opposite walls of the pleura or at other locations within the pleural space.

Tracheo-bronchiolar epithelium. As previously indicated, tracheo-bronchiolar (i.e., non-respiratory) epithelium is composed primarily of ciliated, columnar cells that are 10 to 15 μm thick. Although some report that Clara cells serve as progenitor cells for tracheo-bronchiolar epithelium (Finkelstein et al. 1997), others report that both Clara cells and bronchiolar epithelium are proliferation competent (Nehls et al. 1997). It appears that the identity and location of the progenitor cells for Clara cells are not currently known.

4.4.4 Implications

The potential implications of the above observations concerning lung structure and cytology are:

- that the thicknesses of Type I epithelial cells, endothelial cells, and the interstitium in the alveolar septa are all very small relative to the lengths of the putative asbestos fibers that contribute to disease;
- that an entire alveolus is only 300 μm across and a typical Type I cell is 46 μm in radius by <1 μm thick;
- that distances across alveolar septa are only on the order of a few μm and such septa contain both the endothelium and the interstitum. Thus, the distances that have to be traversed to get to these structures are also small relative even to the length of a fiber;
- that the alveolar septa and the walls of the alveolar ducts fold during respiration, which may provide mechanical forces that facilitate movement of fibers into and through the alveolar epithelium;
- that Type I epithelium do not proliferate so they cannot be the cells that lead to cancer. It is the Type II epithelial cells (and potentially macrophages, basal cells, or endothelial cells) that contribute to cancer in the pulmonary portion of the lung. Type II cells eventually terminally differentiate to Type I cells;
- that other cells in the lung that have variously been reported to be proliferation competent (so that they potentially serve as target cells for the induction of cancer) include Clara cells and bronchiolar epithelial cells;
- that the distance from the most distal airways and alveoli to the pleura is small relative to the lengths of a fiber; and
- that mesothelial cells are proliferation competent and thus serve as potential targets for the induction of cancer.

5.0 THE ASBESTOS LITERATURE

This is a description of the common types of studies in the asbestos literature and an overview of the sources of potential uncertainty typically associated with each. Such limitations must be considered when drawing conclusions from these studies and, more importantly, when deriving inferences based on cross-study comparisons. Throughout this document, we have endeavored to identify the major sources of uncertainty in the studies we examined and have endeavored to account for such uncertainties during our evaluation and interpretation of study results.

The types of studies available for examining relationships between risk and asbestos exposure include human epidemiology studies, human pathology studies, a broad variety of animal studies, and a broad variety of *in vitro* studies in both tissue cultures and cell-free systems. To properly compare and contrast the results from such studies:

- the method(s) employed for asbestos characterization in each study need to be reconciled;
- the procedures employed for evaluating study endpoints need to be compared and contrasted;
- the relationship between the route of exposure employed in each type of study and the exposure route of interest (i.e., human inhalation) needs to be examined; and
- other major, study-specific sources of uncertainty need to identified and addressed.

Among study conditions and procedures that must be considered before evaluating study conclusions, it is particularly important to address the analytical methodologies employed to characterize the nature of exposures (or doses) in each study and such considerations are common to virtually all of the various types of studies of interest.

As indicated in Section 4.3, the only instrument capable of completely delineating asbestos structure-size distributions is TEM (or TEM combined with other techniques). Thus, for example, conclusions regarding variations in biological effects due to differences in such things as fiber size must be viewed with caution when fiber sizes are characterized using only PCM, SEM, or other, cruder analytical techniques. Similarly, the ability to adequately determine fiber mineralogy (fiber type), particularly of what may be minor constituents of various dusts or bulk materials, also depends strongly on the instrumentation employed for analysis as well as the strategy for sampling and for conducting the actual structure count. All of these factors must be considered.

Not only does the specific instrumentation (analytical technique) that is employed in an asbestos measurement affect the outcome of that measurement, but the particular method employed to guide the measurement affects the outcome. As previously indicated (Section 4.3), details concerning the definition of structures to be included in a count, the strategy for counting, and the minimum number of specific types of structures to be included in a count (all features that

vary across analytical methods) affect the precision with which fiber concentrations (particularly of longer and thinner fibers) are delineated. It is not uncommon, for example, that asbestos concentrations measured in the same sample may vary by several orders of magnitude, due simply to a difference in the analytical method employed for the analysis (even when the same analytical instrument is employed, see Section 4.3).

Other important sources of uncertainty tend to be study-type specific and are thus addressed separately below.

5.1 HUMAN EPIDEMIOLOGY STUDIES

A good overview of the kinds of limitations that contribute to uncertainty in the available epidemiology studies was presented in the Health Effects Assessment Update (U.S. EPA, 1986). As described in Appendix A of this document, while evaluating exposure-response factors derived from the human epidemiology studies, an attempt was made to address most of the major sources of uncertainty commonly associated with such studies, which are described briefly below.

Epidemiology studies, which track the incidence of disease (or mortality) within a defined group (cohort) sharing comparable exposures, have been performed on cohorts of workers exposed to asbestos and other mineral fibers in a variety of occupational and environmental settings. Among these, studies that include quantification of exposures are particularly useful for evaluating exposure-response relationships and deriving risk factors.

Generally, the most severe limitations in an epidemiology study involve the exposure data. Both estimates of the level of exposure and determination of the character of exposure are affected by such limitations. Regarding the character of exposure, because exposure measurements from most of the available quantitative epidemiology studies are based on MI measurements or PCM measurements, detailed characterization of the size distribution or the mineral type of fibrous structures (particularly of minor constituents) that contributed to exposure in such studies is generally lacking (Appendix A). This is particularly important because of the evidence that neither MI nor PCM are capable of providing measurements that remain proportional (across study environments) to the biologically-relevant characteristics of an asbestos dust (Berman et al. 1995). This limits the ability both to compare results across the existing studies and to extrapolate such results to new environments for which risks need to be estimated. At the same time, effects on the ability to observe exposure-response trends within a single study are not typically impaired.

Samples collected prior to the mid-1960s were often analyzed by measuring total dust in units of millions of particles per cubic foot (mpcf) using impingers or thermal precipitators. A description of the relative strengths and weaknesses of these techniques is provided in Section 4.3. The fibrous portion of the dust was not monitored. Impinger measurements are sometimes related to fiber counts (based on PCM) using side-by-side measurements of total dust and fiber counts collected during a relatively brief period of time (e.g., Dement et al. 1983a; McDonald et al. 1980b). However, the correlation between fiber counts and total dust is sometimes poor within a plant (i.e., a single study environment) and generally poor between plants (see, for example, U.S. EPA 1986). Thus, conversions based on limited sets of paired

measurements are of questionable validity. In some studies (e.g., McDonald et al. 1983b) the only available measurements are MI measurements (in mpcf) and these have been related to f/ml by PCM using conversion factors derived in other plants, which raises further questions concerning validity.

Even if all measurements could be adequately converted to PCM, this may still not be adequate for assessing risk in a manner that allows extrapolation across exposure environments or studies. Comparing exposure-response factors derived in different exposure environments (or extrapolating to new environments to predict risk) requires that asbestos measurements reflect the characteristics of asbestos structures (size, shape, mineralogy) that determine biological activity. If surrogate measures are employed (e.g., measures of asbestos structures displaying characteristics other than those that determine biological activity), there is no guarantee that concentrations of such surrogate measures and the true biologically active structures will remain proportional from one environment to the next. As a consequence, the relationship between exposure (measured by surrogate) and risk may not remain constant from one environment to the next. Importantly, several studies suggest that PCM may, at best, be no more than a surrogate measure (see, for example, Berman et al. 1995). Moreover, the technique was adapted to asbestos in an *ad hoc* fashion with only limited thought given to biological relevance (Walton 1982).

Use of surrogate measures of asbestos exposure may be less of a problem within a single exposure environment (where airborne asbestos structures likely have been generated in a similar manner from similar source material). Thus, surrogate measures of asbestos exposure may remain approximately proportional to the true biologically active structures, which suggests why monotonically increasing exposure-response relationships have likely been observed with PCM-measured concentrations *in single exposure environments*. In different exposure environments, however, the distribution of fiber sizes and types of airborne asbestos structures are likely different, since they are generated in different processes from different source material. There is thus little reason to expect surrogate measures of exposure to remain proportional across such environments.

None of the published epidemiology studies incorporate TEM measurements of asbestos and such measurements are not widely available in occupational settings (Appendix A). However, TEM is the method currently used (and recommended) to assess exposure in environmental settings, due both to questions concerning biological relevance (Berman et al. 1995 and addressed in detail in Chapter 6) and to problems with measuring environmental asbestos concentrations by PCM (Section 4.3).

In some cases, the limited exposure characterization presented in specific epidemiology studies can be augmented by pairing such studies with published TEM characterizations of dusts from the same or similar exposure settings, to the extent the appropriate supplemental studies are available. In fact, this is the procedure adopted in this document to adjust the existing risk factors to exposure indices that are thought to better relate to biological activity (described in detail in Section 7.4). Such an approach is limited, however, to the extent that the published asbestos characterizations actually represent exposure conditions in the corresponding epidemiology studies. To the extent reasonable, the limitations of this approach have been

addressed in this study by assigning and incorporating additional factors into the calculation of uncertainty intervals (defined in Appendix A) that are associated with the adjusted potency factors.

Regarding levels of exposure in the epidemiology studies, in most cases, air measurements were collected only infrequently and measurements may be entirely lacking from the earliest time periods, when exposures may have been greatest. In such cases, exposures are typically estimated either by extrapolation from periods when measurements are available or by expert judgement based on personal accounts and records of changes in plant operations, industrial hygiene procedures, air standards, etc. Moreover, the majority of exposure measurements used in these occupational studies are based on area (ambient) rather than personal samples. Typically, only a few areas of a plant have been sampled so that levels in other areas must be approximated using expert judgement by persons familiar with operations at the plant.

It is difficult to judge the degree that available asbestos concentration measurements are representative of actual exposures in the existing studies. In some cases, it seems likely that operations were shut down or otherwise modified in preparation for sampling. Likewise, in some operations there are brief episodes of very intense exposure and it is questionable whether such episodes are adequately represented in the available data.

Most of the asbestos measurements used in the published epidemiology studies were collected for insurance or compliance purposes. They were not intended to provide representative estimates of the direct level of exposure to workers. Some of the published epidemiology studies lack any direct exposure data. For example, exposures were estimated for the cohort studied by Seidman (1984) based on conditions simulated many years later in a similar plant to the one from which Seidman studied the original cohort. In fact, the equipment in the plant from which Seidman obtained exposure estimates came originally from the plant where Seidman studied the workers; it was purchased and moved. Recently, an epidemiology study was also completed for a cohort working at that new plant (Levin et al. 1998).

In addition to problems with the actual analysis of asbestos concentrations, individual exposures are generally estimated in the existing epidemiology studies by relating ambient asbestos measurements to job descriptions and integrating the duration of exposure over the recorded time that each worker spent in each job category. However, sometimes there are no records of specific areas in which an employee worked, so that work areas must be assumed based on job title. Some types of workers (e.g., maintenance workers) may have spent time in many different areas of a plant so their exposure varies from what might otherwise be assumed.

Although the greatest problems with the data in existing epidemiology studies likely lies within the estimates of exposure, problems with disease-response data also exist. Mesothelioma is rare and this disease may have been under-reported as a cause of death in older studies. This is probably less of a problem in more recent studies, since the association of mesothelioma with asbestos exposure is now well known. In fact, the opposite tendency (over-reporting) may now be occurring because of increased sensitivity by examiners (an asbestos worker with mesothelioma is now more likely to be eligible for compensation). Some studies have re-diagnosed causes of death from all of the available data (e.g., Selikoff et al. 1979); however,

this creates the problem of lack of comparability to control populations (for which such re-diagnosis is not generally performed).

The choice of an appropriate control population is also an important consideration. Local cancer rates may differ substantially from regional or national rates and the choice of an appropriate control is not always clear. A related problem is the lack of smoking data in many of the studies. Because of the interrelation between smoking and asbestos in lung cancer, errors could occur in lung cancer risk estimates if the smoking patterns of the cohort are substantially different from those of the control population.

In some of the studies, a substantial portion of the population is lost to follow-up (e.g., Armstrong 1988), and this adds additional uncertainty to the analysis. Also, the effect of exposure may be inaccurately evaluated if the follow-up of the population is too brief. This may be a limitation, for example, of the Levin et al. (1998) study.

Another problem frequently associated with these studies is that available data are not reported in a form that is well-suited to risk assessment. The EPA lung cancer model, for example, requires that exposure be estimated as cumulative exposure in f/ml·years excluding the most recent 10 years (U.S. EPA 1986, also described Section 7.2); generally the data are not published in this form. The data are also frequently not available in a form that permits study of the shape of the lung cancer exposure-response curve, so it is not possible to determine how well the EPA model describes the data. The reporting of the mortality data for mesothelioma is generally even less appropriate for risk assessment. Ideally, what is needed is the incidence of mesothelioma subdivided according to exposure level, age at beginning of exposure, and duration of exposure (U.S. EPA 1986, also described in Section 7.3). Such data are almost never available in published studies and crude approximations must be made to account for this lack of information.

It is important to understand the type and magnitude of effect that each of these sources of uncertainties are likely to have on the distribution of potency estimates derived from the set of available studies for lung cancer and mesothelioma, respectively. Some of the above-described limitations likely introduce random errors that simply decrease the overall precision of a potency estimate. However, other types of limitations may cause systematic errors in particular studies, which potentially bias the potency estimate either high or low. Some of the limitations may only affect between-study comparisons and some may introduce a systematic bias between either industry types or fiber types. Examples of some of these types of variation are provided in Section 7.1.

We also note that, although individual estimates of potency factors from individual studies may be highly uncertain, by combining results across multiple studies while properly addressing such uncertainties, it may be possible to draw conclusions with greater precision than reasonable for any individual study. This is the essential advantage of the type of meta analysis discussed in this document (see Chapter 7).

5.2 HUMAN PATHOLOGY STUDIES

Human pathology studies provide a characterization of disease morphology and correlations between causes of death and the types of asbestos fibers retained in the lungs and other bodily tissues. These studies generally involve microscopic examination of tissue samples for indications of morphologic changes characteristic of disease and/or microscopic examination of digested tissue specimens to characterize the mineral fibers extracted from such tissue.

The results of human pathology studies need to be evaluated carefully by addressing effects that are attributable to:

- the way tissue samples are fixed for preservation;
- the way tissue samples are prepared for analysis (e.g., ashing, bleach digestion, digestion in alkali, or some combination);
- the choice of methods employed for characterization of asbestos; and
- the choice of locations within tissues from which samples are collected for analysis.

Because tissue samples obtained from deceased individuals are typically stored for long periods of time before they may be analyzed as part of a human pathology study, such samples are commonly fixed by treatment with chemical preservatives prior to storage. However, Law et al. (1991) studied the effects of two common fixatives (Karnovsky's fixative and formalin fixative) on asbestos fibers and concluded that such fixatives degrade and dissolve asbestos fibers (including both chrysotile and crocidolite) at measurable rates. Therefore, particularly for samples that are stored "wet" (as opposed, for example, to storage in paraffin blocks), the concentrations and character of the tissue burden of asbestos may be altered during storage. Even for studies in which relative (as opposed to absolute) concentrations are being compared, alterations associated with preservation may limit the ability to make such comparisons, particularly among samples stored for widely disparate periods of time or stored using widely disparate procedures.

Fiber concentrations in tissue samples have also been shown to vary as a function of the method employed for preparing such samples for analysis. Historically, samples that are digested in bleach or alkali have tended to exhibit lower recovery of asbestos fibers than samples that are ashed. However, more recent studies suggest that improving technique has narrowed these differences so that this is no longer a major consideration . Thus, when comparing results across studies, due consideration needs to be given for the time frame during which such studies were conducted and the comparability/differences in the techniques employed for tissue sample preparation.

Once prepared, both the character and the concentration of the tissue burden measured in a tissue sample will also depend heavily on the particular analytical method employed to characterize asbestos and differences attributable to such techniques must be reconciled before measurements across samples or conclusions across studies can be reasonably compared. A more detailed

description of the effects attributable to asbestos measurement was presented in the previous section on human epidemiology studies (Section 5.1) and the same issues obtain for human pathology studies. Unless measurements are made using comparable instrumentation with comparable methodology, comparisons across such measurements can be very misleading.

Perhaps the biggest limitation hindering the kinds of evaluation that can be conducted based on human pathology studies is that due to the strong dependence of asbestos concentrations on the specific location within a tissue from which a sample is obtained. Numerous authors have reported that asbestos is non-uniformly distributed in lung parenchyma and other tissues following exposure (see, for example: Bignon et al. 1979; Davis et al. 1986a; Pooley 1982). The incidence of lesions and other pathological effects attributed to asbestos exposure correspondingly exhibit a non-uniform distribution.

For lung tissue samples (which tend to be among the primary interests in human pathology studies) the relationship between sample location and asbestos concentration is particularly important. To sample deep lung tissue reproducibly, it has been shown necessary to select a specific section of lung parenchyma from a defined portion of the bronchio-alveolar tree. Pinkerton et al. (1986) showed that the deposition of asbestos in the lungs is an inverse function both of the path length and the number of bifurcations between the trachea and the site. Thus, analyses of samples from different animals of the same species can only be compared meaningfully if the samples are collected from identical locations in the bronchio-alveolar tree. Similar, nonuniform depositional patterns have also been observed in humans (Raabe 1984). Furthermore, due to the complex branching and folding pattern of the lung, adjacent sections of lung parenchyma frequently represent disparate portions of the bronchio-alveolar tree (Brody et al. 1981; Pinkerton et al. 1986). Consequently, lung burdens derived even from adjacent samples of lung parenchyma can show broadly varying concentrations (differing by orders of magnitude).

Unfortunately, however, tissue samples that are available for analysis in support of a human pathology study are typically "opportunistic" samples, which means that they were selected and stored for an entirely different purpose than the study at hand and, although there may sometimes have been attempts to sample comparable locations across lungs in a general way, this is not adequate for assuring that comparable portions of the respiratory tree are being sampled. It is therefore seldom possible to address the effects of sample location directly. Consequently, comparisons of tissue burden concentrations across samples from different individuals in a human pathology study are at best qualitative and may only be useful when averaged over large numbers of individuals and only when large differences in concentrations (several orders of magnitude) are being distinguished. Moreover, because the parts of a tissue that undergoes morphologic changes induced by asbestos typically corresponds to the parts of a tissue where asbestos burdens are the highest, even comparison of morphologic effects across tissue samples requires proper consideration of the effects of the locations from which such tissue samples were derived.

5.3 ANIMAL STUDIES

In animal studies, members of one of various species (generally rodents) are exposed to measured doses of size-selected mineral fibers and the resultant biological responses are monitored. Animals may be dosed either by inhalation, ingestion, intratracheal installation, implantation, or injection (U.S. EPA 1986). Such studies are conducted for several purposes. As with human pathology studies, animal pathology studies are those in which the transport of asbestos structures is tracked through the various organs and tissues of the animal and the attendant cellular and molecular changes are characterized. In parallel with quantitative epidemiology studies, animal dose/response studies track the incidence of disease among a population that has been exposed in a controlled manner. One of the advantages of animal dose/response studies over epidemiology studies is that exposures are controlled and can be well characterized. The major disadvantage is that there are many uncertainties introduced when extrapolating the results of animal data to predict effects in humans. Therefore, attempts to adapt such things as animal-derived dose-response factors to humans are not generally recommended.

As with human epidemiology and pathology studies, the validity of conclusions drawn from animal studies depends strongly on the techniques and methods used to characterize and quantify asbestos structures either in the delivered dose or in the tissues of the dosed animals (see Section 5.1). The ability to reconcile conclusions derived from many animal studies with the rest of the asbestos literature is limited because SEM was commonly employed to measure asbestos in animal studies, but not other kinds of studies. Even many of those studies in which TEM was employed for asbestos analysis suffer from use of non-standard methods that cannot be easily reconciled with the more traditional TEM methods, particularly because such specialized methods are seldom adequately documented to allow comparison.

As with human pathology studies, the location of a tissue sample excised for analysis is a critical factor that also governs the quality of an animal pathology study (Section 5.2). However, one potential advantage frequently available in animal pathology studies over human studies is the ability to carefully identify and select the precise tissue samples to be analyzed. The extent that a particular animal pathology study exploits this capability can affect the overall utility of the study. Thus, such issues need to be addressed carefully when evaluating and comparing the results across animal pathology studies.

The route of exposure employed in a particular animal study is also important to consider. Each of the routes of exposure commonly employed in these studies (inhalation, ingestion, intratracheal installation, and injection or implantation) delivers different size fractions of asbestos to a target tissue with varying efficiencies. For example, injection or implantation studies deliver 100% of all size categories of structures to the target tissue. However, the efficiency that each size category is delivered by inhalation is a function of the aerodynamic properties of the asbestos structures and the air flow characteristics of the lungs (see, for example, Yu et al. 1991, 1994). Thus, the relationship between dose and exposure depends upon the route of exposure employed. Importantly, because the ultimate goal is to understand the effects that inhaled fibers have on humans, differences between the character of the delivered dose in an animal study and the character that such a dose would have, had it originally been inhaled, typically need to be addressed.

Regarding the measurement of health effects, many of the results in animal studies suffer from a lack of statistical significance because of small numbers of observed tumors. Consequently, trends cannot be established conclusively.

For animal inhalation studies, meaningful comparison of the relative deposition of asbestos dusts across species is not direct. To extrapolate results across species, the detailed differences in the physiology of the respiratory tracts between the species need to be addressed (Section 4.4). However, if measurements are available for both species, differences in physiology are addressed, and the manner in which tissue burdens are analyzed is considered, it may be possible to compare relative tissue doses (mass of asbestos per mass of tissue) across species.

5.4 *IN VITRO* STUDIES

A broad range of *in vitro* studies provide useful insight on the effects of asbestos. These include, for example, studies in cell-free systems (which have been used to evaluate such things as asbestos dissolution rates or the kinetics of free radical formation on the surface of asbestos fibers) and studies of the effects of asbestos on cultures of a broad variety of cell types and tissues.

As with other studies, the potential limitations and sources of uncertainty associated with *in vitro* studies need to be considered when evaluating the validity of study results or comparing such results to those of other studies, particular studies of varying type. Also, as with other studies, among the primary sources of uncertainty that need to be addressed for *in vitro* studies is the manner in which asbestos doses are characterized and quantified (Section 5.1). For *in vitro* studies (as with animal studies dosed by routes other than inhalation), this also extends to the need to consider the relationship between the character of the asbestos dose applied *in vitro* and the character that a similar exposure might possess following inhalation exposure *in vivo* (Section 5.3). This can be particularly problematic for studies in tissue cultures because it is not clear how the application of a suspension of fibers (with known concentration) to a dish containing cultured cells can be related to doses that reach corresponding tissues following administration to whole animals.

In vitro studies, of necessity, represent isolated components of living systems observed under conditions that may vary radically from those under which such components operate *in vivo*. Consequently, the behavior of such components may also vary radically from the behavior of the same components *in vivo*. Therefore, additional study-specific considerations (concerning the design of a study and the conditions under which a study is conducted) also need to be addressed before evaluating the validity or relevance of the results from an *in vitro* study to what might otherwise be observed in a whole animals. Examples of such considerations include:

For cell-free systems

- whether conditions under which the study is conducted are sufficiently similar to conditions *in vivo* to expect that the observed effect is likely to occur *in vivo*; and

- if the observed variables describing the nature or magnitude of the effect are also likely to reflect what may occur *in vivo*;

For tissue cultures

- whether conditions under which the study is conducted are sufficiently similar to conditions *in vivo* to expect that the observed effect is likely to occur *in vivo*;

- whether responses by specific tissues or cells in culture are likely to behave similarly *in vivo* where their behavior may be suppressed, enhanced, or modified in some other manner due to additional stimuli provided by responses of other tissues and cells that are components of a complete organism but that may be lacking in culture; and

- whether the conditions required to establish and maintain a tissue culture for experimentation sufficiently alters the characteristics and behavior of the cells being studied to minimize the relevance of results from such a study to conditions *in vivo*.

Among the most important examples of the last consideration relates to the general need to create immortalized cells to maintain tissue cultures. Thus, questions must always be raised concerning whether the alterations required to create immortalized cells for culture (from what are normally mortal cells *in vivo*) also alter the nature of the responses being studied.

Note, many studies in the current literature also incorporate combined aspects of several of the four general study types described in this chapter. For these studies, a corresponding combination of the considerations described must therefore be addressed when evaluating such studies and comparing their results with inferences derived from the rest of the literature.

6.0 SUPPORTING EXPERIMENTAL STUDIES

To evaluate the plausibility of cancer risk models for asbestos it is useful to examine the current state of knowledge regarding (1) the mechanisms that facilitate the transport of asbestos to the various target tissues of interest (i.e., the lungs and mesothelium) and (2) the mechanisms that contribute to the development of cancer in these target tissues. Accordingly, a review of the relevant literature is provided in this chapter to assure that the quantitative analysis in Chapter 7, and the proposed approach for assessing asbestos-related risks in Chapter 8, are qualitatively consistent with general implications from the broader literature. This review, although extensive, is not exhaustive. A much larger number of studies was reviewed than are actually cited. Studies that are not cited are largely confirmatory or redundant. In selecting studies for review, special effort was expended to assure that opposing views (particularly for controversial issues) were adequately represented.

Although much progress has been made over the last decade toward elucidating the fiber/particle mechanisms that contribute to transport and subsequent cancer induction, at least two critical data gaps remain:

- no one has yet been able to track a specific lesion induced by asbestos in a specific cell through to the development of a specific tumor. There have been experiments that show altered DNA and other types of cellular and tissue damage that are produced in association with exposure to asbestos. Other studies have demonstrated that various tumors of the kinds that result from asbestos exposure exhibit patterns of DNA alteration (or other kinds of cellular damage) that are sometimes (but not always) consistent with the earlier cellular changes associated with asbestos exposure. There are also studies that show that exposure to asbestos can lead ultimately to development of tumors. However, these types of studies have yet to be linked; and

- the specific target cells that serve as precursors to tumors in various target tissues are not known with certainty.

Because of the first of the above limitations, researchers have tended to report on a broad range of tissue and cellular effects induced by asbestos that may lead generally to various kinds of cellular damage or injury. Cytotoxicity, for example, is one of the endpoints typically tracked as a marker for asbestos-induced injury. However, not all of these effects necessarily contribute (either directly or indirectly) to the development of cancer. Therefore, one of the goals of the following discussion is to distinguish among effects that likely contribute to the development of cancer from those that are less likely or unlikely to contribute. Of course, delineating such distinctions are subject to the limitations of the current state of knowledge.

In addition, because the relative effects of fiber size, shape, and mineralogy need to be elucidated to better indicate how asbestos concentrations should be characterized to support risk assessment, studies that address these topics are highlighted. Of particular interest are studies that (1) contrast the effects of different sized fibers, (2) contrast the effects of fibers and non-

fibrous particles of similar mineralogy, and (3) contrast the effects of fibers of comparable morphology (size and shape), but differing mineralogy.

The types of studies that have contributed to the state of knowledge of the effects of asbestos (in addition to the human epidemiology studies that are evaluated in Chapter 7) include:

- whole animal inhalation studies;
- whole animal instillation studies;
- whole animal injection, implantation studies;
- human pathological studies;
- *in vitro* studies in cell cultures; and
- *in vitro* studies in cell-free systems.

Depending on the outcome(s) monitored, the animal studies may alternately be categorized as retention studies, histopathology studies, or dose-response studies.

Each type of study possesses certain advantages and exhibits certain limitations, which have previously been described (Chapter 5) along with descriptions of the nature of each of these study types. In addition to the advantages and limitations that are attributable to the type of study, the quality of the characterization of asbestos (or other particulate matter) determines the utility of the study for addressing issues associated with fiber morphology and mineralogy. Unfortunately, for many published studies, both the characterization of the asbestos (or other particulate matter) and descriptions of the manner in which such materials were handled are insufficient to establish the detailed morphology or mineralogy. Such limitations need to be considered when comparing across study results or evaluating the validity of study conclusions.

The rest of this chapter is divided into separate sections that address the set of factors that have been previously identified (Chapter 3) as those that determine the biological activity of inhaled asbestos (which is the exposure route of primary concern for humans). These are:

- the extent that asbestos structures are respirable and the pattern of deposition of inhaled structures;
- the extent that deposited structures are subsequently cleared or degraded;
- the extent that deposited structures are transported or migrate to the various target tissues; and
- the extent that retained structures induce a biological response in each target tissue.

6.1 FACTORS AFFECTING RESPIRABILITY AND DEPOSITION

Discounting systemic effects resulting from other forms of exposure, factors affecting respirability are common to all of the toxic endpoints associated with asbestos exposure considered in this study (asbestosis, lung cancer, and mesothelioma). Moreover, respirability is common to the factors affecting the toxicity of inhaled, insoluble particles in general. To be

respirable, an inhaled particle must pass the blocking hairs and tortuous passageways of the nose and throat and be deposited in the lungs. Particles deposited in the naso-pharyngeal portion of the respiratory tract are not considered respirable.

Not all of the inhaled particles that reach the lungs will be deposited. Small particles may not impact lung surfaces during inhalation and are subsequently exhaled. Once a particle impacts on a surface, however, it is likely to remain because the surfaces of the lungs are wetted with a surfactant (Raabe 1984).

Adverse health effects potentially result when particles that are deposited in the lungs remain in contact with the tissues in the lung for a sufficient period of time to provoke a biological response. To affect the mesothelium, an offending particle may also need to migrate or be transported from the lung to this surrounding tissue. However, due to the proximity of the mesothelium to peripheral portions of the lung parenchyma (which include locations where particles are typically deposited), it is also possible that diffusable molecules produced in lung tissue in response to deposited particles can have an adverse effect on the mesothelium (see, for example, Adamson 1997). Such effects are considered among the mechanisms of disease induction addressed in the discussion of biological responses (Section 6.3).

The interplay between deposition and removal (clearance) of inhaled particles is an important determinant of biological activity and separating the influence of these two processes in the pathology of asbestos-induced disease is difficult. The term "retention" is used here to represent the fraction of particles remaining in the lungs beyond the time frame over which only the most rapid removal processes (i.e., muco-ciliary clearance) are active. The factors affecting retention are addressed further in Section 6.2.

Published inhalation studies divide the respiratory tract into three units (see, for example, Raabe 1984). The naso-pharyngeal portion of the respiratory tract extends from the nares in the nose through the entrance to the trachea. The tracheo-bronchial portion of the respiratory tract includes the trachea and all of the branching bronchi down to the terminal bronchioles. The respiratory bronchioles and the alveoli, which are collectively referred to as the "deep lung", are the bronchio-alveolar (or pulmonary) portion of the respiratory tract. For a more detailed description of the features of the respiratory tract, see Section 4.4.

The dimensional requirements for respirability have been studied and reviewed in several studies (see, for example, Raabe 1984 or U.S. EPA 1986). A more recent review is also presented by Stober et al. (1993). Much of the data reviewed in these studies is based on earlier studies in which researchers exposed animals or human volunteers to a series of monodisperse spherical particles (although Stober et al. also reviews fiber experiments). In this manner, the impact of the diameter of spherical particles on respirability was elucidated. The respirability of fibrous materials (such as asbestos) tends to be described in terms closely associated with those employed for spherical particles, but with adjustments for density and shape. Importantly, because respirability is a mechanical process: the size, shape, and density of a particle (or fiber) determine its respirability along with the morphometry of the airways through which the particle passes (Stober et al. 1993). Other than affecting the particle's density or the distribution of fiber shapes, the chemical composition (mineralogy) of a particle (or fiber) does not influence respirability.

The respirability of particles and fibers by humans, and a variety of other mammals of experimental interest, has also been the subject of increasingly sophisticated modeling efforts (Stober et al. 1993). The latest refinements of such models predict particle deposition with a degree of accuracy that is beyond what can be validated with existing, experimental data. The application of several of these models to asbestos (and other fibrous materials) are considered throughout this chapter. However, a detailed overview of the state-of-the-art of such modeling is beyond the scope of this document. Such an overview is presented by Stober et al. (1993).

6.1.1 Respirability of Spherical Particles

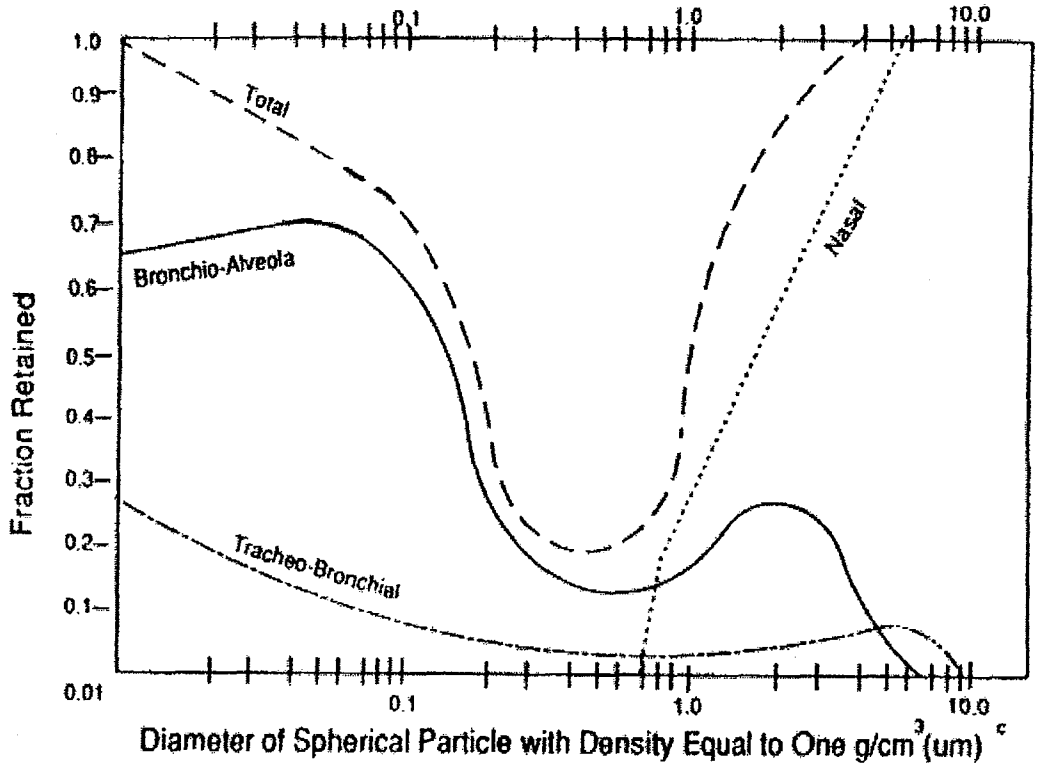
Spherical particles larger than 10 μm in diameter are considered non-respirable because virtually all particles in this size range are trapped in naso-pharyngeal passageways and blocked from entering the lungs. As the diameter of the particles fall, an increasing fraction traverses the nose and throat and may be deposited in the lungs. About half of particles 5 μm in diameter are blocked before entering the lungs. Virtually all particles smaller than 1 μm enter the lungs, although other factors determine whether they are in fact deposited or simply exhaled. Figure 6-1 (Raabe 1984) is a representation of the relative deposition in the various compartments of the respiratory tract as a function of particle diameter.

Within the lungs (Figure 6-1), the greatest fraction of respirable particles (over the entire range of diameters down to <0.01 μm) are deposited in the deep lung (the broncho-alveolar portion of the respiratory tract), primarily at alveolar duct bifurcations (see, for example, Brody et al. 1981; Davis et al. 1987; Johnson 1987; Sussman et al. 1991a). These studies also indicate that biological responses appear to be initiated where deposition is heaviest. Generally, the fraction of particles deposited in the deep lung increases regularly with decreasing diameter until a maximum of 60% deposition in the deep lung is reached at about 0.1 μm diameter.

As indicated in Figure 6-1, a transition occurs at particle diameters between 0.5 and 1 μm. For particles in this range and smaller, deposition in the deep lung competes primarily with deposition in the tracheo-bronchial tree and with exhalation; smaller particles have an increasing probability of being exhaled without ever impacting the surface of an air passageway. For particles larger than this transition range, broncho-alveolar deposition is limited chiefly by the fraction of particles that are removed from the air stream prior to reaching the deep lung (either by deposition in the naso-pharyngeal or the tracheo-bronchial portions of the respiratory tract).

The transition between naso-pharyngeal competition with deep-lung deposition and competition from other removal processes is important because, during mouth breathing, a process that bypasses the tortuous pathways of the nose and throat, it has been observed that larger particles (up to several micrometers in diameter) may be deposited in the deep lung (Raabe 1984). Studies of the effects of mouth breathing are also reviewed by Stober et al. (1993). Because most people spend at least small amounts of time mouth breathing, especially during exertion or while snoring, this mechanism for allowing larger particles to settle in the deep lung should not be ignored.

Figure 6-1. Fractions of Respirable Particles Deposited in the Various Compartments of the Human Respiratory Tract as a Function of Aerodynamic Equivalent Diameter[a,b]



[a]Source: Raabe 1984
[b]Assumes a typical tidal value of 1,450 cm^3 and a rate of 15 breaths a minute
[c]Aerodynamic equivalent diameter

Confidential: Need Permission to Reproduce this Figure

A diameter of 0.5 μm also happens to represent the transition between the regime where inertial flow becomes the major factor controlling deposition in the lungs and the regime where diffusional flow dominates. Below the 0.5 μm transition, the diffusional diameter becomes more important in determining deposition than the aerodynamic equivalent diameter (defined below).

6.1.2 Respirability of Fibrous Structures

Several authors have investigated the effect of the shape of non-spherical particles (including fibers) on respirability and deposition (see, for example, Harris and Timbrell 1977; Strom and Yu 1994; Sussman et al. 1991a,b; Yu et al. 1995a,b). It has been found that the behavior of non-spherical particles can be related to the behavior of spherical particles by introducing a concept known as the aerodynamic equivalent diameter. The aerodynamic equivalent diameter is the

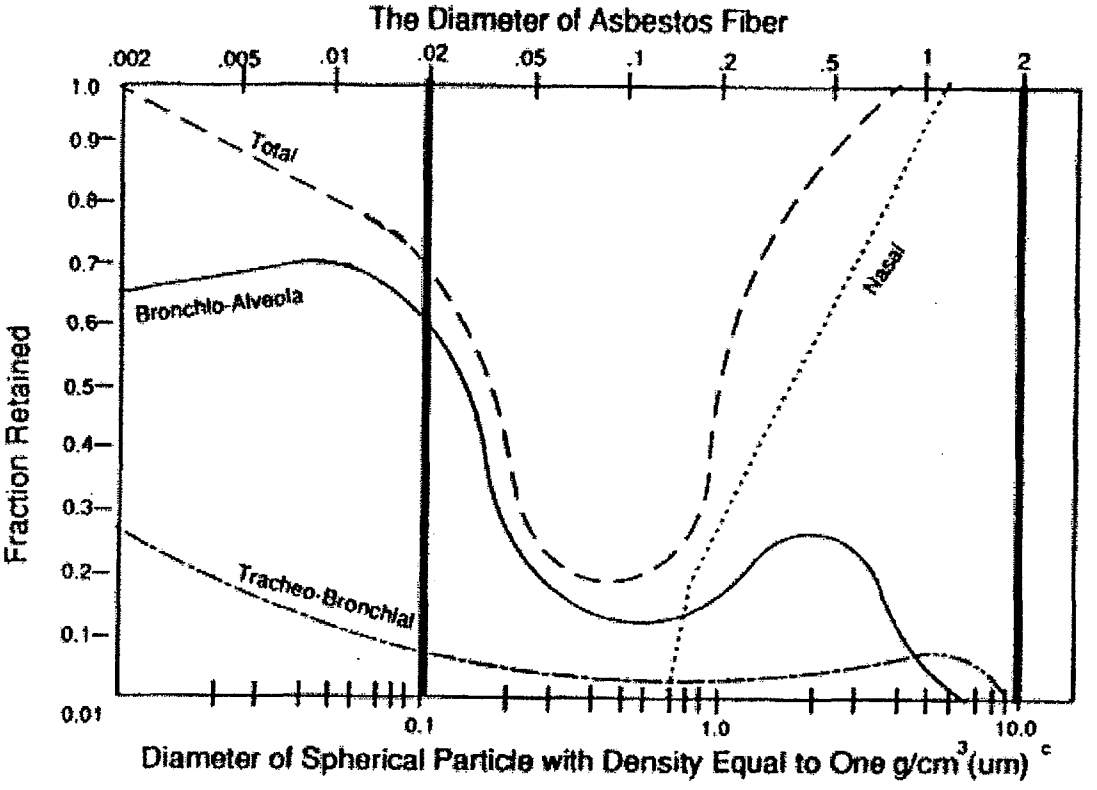
diameter of a hypothetical spherical particle of unit density that would exhibit the same settling velocities and aerodynamic behavior as the real, non-spherical particle of interest. Factors that affect the aerodynamic equivalent diameter are density, true diameter, true length (for elongated particles such as fibers), and the regularity of the particle shape.

Harris and Timbrell (1977) Findings. Because fibrous particles tend to align primarily along the axis of travel under the flow conditions found in the lungs, respirability is predominantly a function of the diameter of a fiber and the effect of length is secondary (Harris and Timbrell 1977). Fibrous structures with aspect ratios (ratio of length to width) >3:1 behave like spherical particles (of similar density) with diameters up to 3 times larger and exhibit only a very weak dependence on length. As previously indicated, however, the aerodynamic equivalent diameter of a fibrous structure must also be adjusted for the effects of density. This is demonstrated in Figure 6-2 where the true diameter of a fiber is graphed on the top horizontal axis against spherical (aerodynamic equivalent) diameters on the bottom horizontal axis. Figure 6-2 is an overlay of Figure 6-1. Note that, to adjust for the density of asbestos, the true diameters listed in the figure have been shifted to the right of where they would appear if the relationship was exactly 1/3 of the aerodynamic equivalent diameter.

Two vertical dashed lines in Figure 6-2 represent effective limits to the range of respirable asbestos. The line on the left side in the figure represents the limiting diameter of the smallest chrysotile fibril (about 0.02 μm true diameter) and thus represents a lower limit to the diameter that is of concern when considering asbestos. The vertical line to the right represents the cutoff where deposition in the deep lung becomes unimportant due to removal of such particles by the naso-pharyngeal passageways. This latter cutoff corresponds to a true fiber diameter of 2.0 μm, which theoretically represents the upper limit to the size of asbestos that is respirable. As indicated in the figure, however, deposition in the deep lung drops precipitously for fibers thicker than about 0.7 μm so that no more than a few percent of asbestos fibers thicker than approximately 1 μm actually reach the deep lung.

Harris and Timbrell (1977) also evaluated the relationship between the overall shape of a particle and the extent of deposition. Over the range of diameters that potentially represent the range of asbestos fibers likely to be encountered, pulmonary deposition decreases with increasing complexity of shape beyond simple cylinders (such as clusters and matrices (see Section 4.2) at the expense of increasing naso-pharyngeal or tracheo-bronchial deposition. This change also becomes increasingly important as the length of the structure increases. For structures <25 μm in length, the difference in deposition between simple fibers and complex clusters or matrices may vary by up to a factor of 2 with the complex structures being more likely to be removed in the naso-pharyngeal portion of the respiratory tract and the fibers more likely to be deposited in the deep lung. At 100 μm lengths, the fraction of complex structures that survive passage through the nose and throat in comparison with simple fibers may vary by a factor of 5. This means that large structures become relatively less respirable as their complexity increases. However, during mouth breathing large clusters and matrices may enter the deep lung.

Figure 6-2. Fractions of Respirable Particles Deposited in the Various Compartments of the Human Respiratory Tract as a Function of the True Diameter of Asbestos Fibers[a,b,d]



[a]Source of original: Raabe 1984
[b]Assumes a typical tidal value of 1,450 cm^3 and a rate of 15 breaths per minute
[c]The relationship between true diameters and aerodynamic equivalent diameters derived from Harris and Timbrell (1977). Diameters adjusted for shape and density of asbestos fibers.
[d]Aerodynamic equivalent diameter

Confidential: Need Permission to Reproduce this Figure

When all of the factors that Harris and Timbrell (1977) addressed are considered, the efficiency of the deposition of asbestos structures in the deep lung is maximal for short, thin, single fibers (<10 μm in length with a true diameter <0.7 μm). The efficiency decreases slowly with increasing length (up to an effective limit of 200 μm), moderately with increasing complexity of shape, and rapidly with increasing diameter (up to an effective limit of 2.0 μm, true diameter). Thinner fibers, down to the lower limit of the range for asbestos fibers (0.02 μm, true diameter), are deposited with roughly the same efficiency. Approximately 20–25% of the fibers between 0.7 and 0.02 μm in diameter (and <10 μm in length) are deposited in the deep lung.

Sussman et al. (1991a,b) Findings. Based on a series of experiments on human tracheal bronchial casts, Sussman et al. (1991a,b) also developed models of fiber deposition in the human lung. Such experiments are in fact illustrative of several research groups who have developed deposition models based on results from experiments on airway casts (for a review, see Stober et al. 1993).

The results reported by Sussman et al. (1991a,b) appear to be generally consistent with the results reported by Harris and Timbrell (1977) and Yu and coworkers (described below), although the manner in which their results are reported make them somewhat less directly comparable. Briefly, Sussman et al. (1991a,b) report that deposition increases along most generations of the bronchial tree with increasing fiber length and increasing airflow rate for any fixed aerodynamic diameter. This increased deposition efficiency is demonstrated for airway generations at least through the ninth bifurcation and is implied to continue through to airway generations that would be representative of the respiratory (pulmonary) portion of the lung (i.e., airway bifurcations greater than approximately 16 to 22). For definitions and a description of airway generations, see Section 4.4.

Findings of Yu and Coworkers. In a series of studies, Yu and coworkers combined an improved model of human lung physiology (Asgharian and Yu 1988) with a series of more rigorous equations to describe fiber mobility (Chen 1992) and used these to evaluate the deposition of various types of fibrous materials in the lung. The trends indicated in their studies show general agreement with those reported by Harris and Timbrell (1977), but with several notable refinements.

In a study of refractory ceramic fibers (Yu et al. 1995a), a maximum deposition efficiency of 15% is reported for fibers that are approximately 6 μm long and approximately 1 μm in diameter. This is close to the fiber size at which maximal deposition is reported by Harris and Timbrell (1977). As with Harris and Timbrell (1977), Yu et al. (1995a) also report that deposition efficiency decreases precipitously as diameter increases beyond 1 μm and decreases more slowly as diameter decreases below 1 μm. For thinner structures, deposition efficiency increases with both decreasing width and length. As fibers get longer, optimum deposition occurs with decreasing thickness. Thus, for example, a maximum deposition rate of 10% occurs for fibers that are 20 μm long at a thickness of 0.8 μm.

In a study of silicon-carbide whiskers (Strom and Yu 1994), the deposition model is extended to fiber widths as narrow as 0.01 μm. Results from this study indicate that fibers between 0.01 and 0.1 μm in thickness are deposited with a minimum efficiency of 5% up to lengths of approximately 40 μm before efficiency drops below 5%. For thin fibers (thinner than 0.5 μm), shorter fibers tend to be deposited in the deep lung much more efficiently than longer fibers. More than 25% of thin fibers shorter than 1 μm are deposited in the deep lung following inhalation. Strom and Yu (1994) report that the efficiency of deposition in the deep lung of long structures increases substantially during mouth breathing.

Comparing the results reported for refractory ceramic fibers (density=2.7 g/cm^3) and silicon-carbide whiskers (density=3.2 g/cm^3), it also appears that the efficiency of deep-lung deposition increases for thinner and for longer structures as the density of the structures increases. Given the observed density effect, chrysotile fibers that are longer than approximately 6 μm and thinner

than 1 μm would be deposited in the deep lung less efficiently than (denser) amphibole fibers of the same size. However, shorter and thicker chrysotile structures would be deposited somewhat more efficiently than similarly sized amphiboles. This suggests that a greater fraction of the mass of chrysotile that gets deposited in the deep lung will be composed of very short fibers and somewhat longer bundles than the mass fraction of short fibers or longer bundles in the air breathed. Also, to the extent that chrysotile fibers are curved, these would be deposited somewhat less efficiently than straighter (amphibole) fibers of comparable size.

Based on the deposition efficiencies predicted by Yu and coworkers, fibrous structures that reach the deep lung in humans are effectively limited to those thinner than approximately 1 μm. Given that fibrous structures have traditionally been defined as particles exhibiting aspect (length to width) ratios >3:1 (Walton 1982), it is clear that only particles shorter than 3 μm could potentially be respirable and still be excluded from the definition of a fibrous structure based on aspect ratio. Therefore, the thickness constraint for all longer structures is best described as a maximum width (rather than an aspect ratio) when defining the range of structures that potentially contribute to biological activity.

Rats versus Humans. Yu and coworkers also modified their models to evaluate the rates that fibrous materials are deposited in rat lungs and compared these with results for humans. Such comparisons have implications for the manner in which results from animal inhalation studies are extrapolated to humans.

Results from Yu et al. (1994) suggest that pulmonary deposition of all fibrous structures with lengths between about 1 and 100 μm and thinner than approximately 1 μm occurs at much higher rates in rats than in humans. Fibers as long as 90 μm are deposited in rat lungs at efficiencies exceeding 20% while fewer than 5% of structures this long are deposited in the pulmonary region of human lungs. In fact, it is only structures between 1 and about 20 μm within a very narrow range of thicknesses (centered around 1 μm) that are deposited more efficiently in the deep lungs of humans than in rats.

Yu et al. (1995a) also indicate that, even when deposition efficiencies are comparable in rats and humans, due to differences in the total lung mass and breathing dynamics across species, the resulting lung burdens (i.e., the mass or number of structures per mass of lung tissue) are 5–10 times higher in the rat than in humans for any given exposure. Lung burden per lung surface area are also higher in the rat than in humans.

To illustrate, assume rats and humans are similarly exposed to a concentration of 0.1 f/cm^3 (100 f/L) of some fibrous material with a length at which both species retain approximately 10% of the fibers inhaled. Table 6-1 then indicates the calculations required to determine the relative rates at which the lung (volume and surface area) burdens in each species would develop.

Table 6-1. Estimation of Lung Volume and Lung Surface Area Loading Rates for Rats and Humans

Species	Body Weight (kg)	Lung Volume (L)	Lung Surface Area (m^2)	Rest Breaths per Minute (bpm)	Tidal Lung Volume (L)
Human	70	5	140	15	1.5
Rat	0.15	0.01	0.4	70	0.0019

Species	Breathing Rate (L/min)	No. Fibers Inhaled per Minute (f/min)	No. Fibers Deposited per Minute (f/min)	Lung Volume Loading Rate (f/L-min)	Lung Surface Area Loading Rate (f/m^2-min)
Human	21.7	2170	217	43.4	1.6
Rat	0.13	13.3	1.3	130	3.3

From Table 6-1, it is clear that rats exposed to comparable airborne concentrations as humans will increase their loading of fibers per volume (or mass) of lung at a rate that is approximately 3 times that of humans (for fibers in sizes that are deposited with 10% efficiency in both species). Similarly, the fiber load per surface area of lung will increase in rats at a rate that is approximately twice that of humans. Moreover, even higher relative mass or surface area loading rates are expected for the rat than shown in the table, due to the greater efficiency with which most fiber sizes are deposited in rat lungs. Data used to compute the loading rates in the table (which are also presented) are derived from Gehr et al. (1993) and supplemented with information from Stober et al. (1993). A more detailed description of this information is provided in Section 4.4.

6.1.3 The Effects of Electrostatic Charge on Particle Respirability

Electrostatic charge has been shown to affect the retention of particles within the lungs (see, for example, Vincent 1985). Since processes that generate airborne particles generally involve some form of abrasion, airborne dust particles frequently exhibit varying degrees of electrostatic charge. Although this potentially leads to variation in the efficiency of particle retention in the lungs as a function of the source of the dust, a detailed relationship between surface charge and retention was not described in this paper. A more detailed and quantitative treatment was developed by Chen and Yu (1993) and the implications of the Chen and Yu model are described below (following discussion of the results of Davis et al. 1988a). Davis et al. (1988a) report that animals exposed to dusts containing fibrous chrysotile, whose surface charge is reduced with a beta minus source, retain significantly less chrysotile than animals dosed with dusts containing particles whose surface charge has not been reduced. However, the magnitude of the difference in the mass of fibers retained is less than a factor of 2, implying that the absolute variation due to this effect may be small. Further research in this area is needed.

Chen and Yu (1993) report that, based on modeling of lung deposition, overall deposition increases with increasing charge density on the particles inhaled. However, due to the pre-

iltering by the naso-pharyngeal and tracheo-bronchial portions of the respiratory tract, the effects of electrostatic charge on deep lung deposition appear to be only slight to modest.

Given the results of the above studies, the overall effects of electrostatic charge on particle deposition in the deep lung appear to be relatively minor. Therefore, such effects do not need to be considered explicitly when evaluating the health consequences of asbestos.

6.1.4 General Conclusions Concerning Particle Respirability

Based on the information provided in the last several sections, it is apparent that in humans:

- deposition of asbestos fibers in the pulmonary portion of the lung occurs primarily at alveolar duct bifurcations;
- electrostatic effects on pulmonary deposition are likely minor;
- fibers that are deposited in the pulmonary portion of the lung are largely thinner than approximately 0.7 μm and virtually all are thinner than 1 μm (except during mouth breathing, when thicker and more complex structures may be respired);
- the length of a fiber has limited impact on respirability up to a length of approximately 20 μm, but the efficiency of deposition of longer fibers decrease slowly with increasing length for longer fibers;
- as the length of the fibers that are inhaled increases, the thinner fibers are deposited with greater efficiency. Thus, the longer the fibers inhaled, the thinner the fibers retained;
- due to differences in density, shorter and thicker chrysotile structures will be deposited more efficiently in the pulmonary portion of the lung than corresponding amphibole structures and longer and thinner amphibole structures will be deposited more efficiently than corresponding chrysotile structures;
- curly chrysotile structures are less likely to reach the pulmonary portion of the lung than straight amphibole (or chrysotile) structures;
- except for a very narrow range of fiber sizes (centered around 6 μm in length and 1 μm in diameter), virtually all size fibers are deposited with greater efficiency in rat lungs than human lungs;
- due to body morphology and the dynamics of breathing, rats exposed to similar air concentrations will accumulate fiber burdens both per mass (volume) of lung tissue and per lung surface area at a rate that is several times the rate such burdens accumulate in humans; and
- the dynamics of fiber lung deposition can now be accurately predicted in great detail using currently available models.

6.2 FACTORS AFFECTING DEGRADATION, TRANSLOCATION, AND CLEARANCE

Degradation and clearance mechanisms compete with deposition to determine the fraction of asbestos that is retained in the lungs. Other (translocation) mechanisms mediate the movement of asbestos from sites of initial deposition to various target tissues within the lung and mesothelium. These factors affect all of the toxic endpoints of interest. Studies indicating the dependence of the various contributing mechanisms on fiber size and mineralogy are highlighted, as well as studies indicating differences between mechanisms in humans and laboratory animals.

The three units of the respiratory tract defined in the last section (naso-pharyngeal, tracheo-bronchial, and bronchio-alveolar units) differ primarily by the types of clearance (and translocation) mechanisms operating in each unit (Raabe 1984). These are summarized in Table 6-2 along with rough estimates of the time frames over which each mechanism may operate (to the extent that such estimates are available in the literature).

Briefly, the structures of the nose and throat are bathed in a continual flow of mucous, which is ultimately swallowed or expectorated. The mucous traps deposited particles and carries them out of the respiratory tract. The air channels of the tracheo-bronchial section of the respiratory tract are lined with cilia and mucous secreting cells. As in the nose and throat, the mucous traps particles deposited in these air pathways and the ciliary escalator transports the mucous up to the throat where it may be swallowed or expectorated. Neither the alveolar ducts nor the alveoli of the pulmonary compartment of the lung are ciliated (inferred from St. George et al. 1993). Therefore, particles deposited in this section of the respiratory tract can only be cleared by the following mechanisms:

- if the deposited particles are soluble, they may dissolve and be transported away from the lungs in blood or lymph; or

- if they are sufficiently compact, they may be taken up by alveolar macrophages and transported outward to the muco-ciliary escalator of the tracheo-bronchial portion of the respiratory tract.

Due to a combination of chemical and physical stresses in the environment of the lung, deposited asbestos structures may degrade by splitting. Longitudinal splitting, primarily of bundles, produces thinner structures and transverse splitting produces shorter structures. In both cases, the number of structures produced may be larger than the number of structures initially deposited.

By changing the size and number of structures that were initially deposited in the lungs, splitting may affect the rates and efficiency with which the various other degradation and clearance mechanisms operate.

Table 6-2. Relative Rates, Half-lives for Particles Cleared by the Varous Operating Mechanisms of a Healthy Lung

Tissue/Lung Regime	Species	Fiber Type	Particle Half-life[a] (days)	Kinetic Order	Mineralogical Effects	Size Effects	Reference
Mechanisms							
(Component Mechanisms)							
Nasal-pharyngeal							
Expectoration and Swallowing	Human		Minimal				
Muco-ciliary Transport	Human	Particles	0.0028	Zero	No Effect	No Effect	Raabe 1984
Tracheo-bronchial							
Muco-ciliary Transport	Human	Particles	0.021-0.21	Zero	No Effect	No Effect	Raabe 1984
Pulmonary (Bronchio-alveolar)							
AM Phagocytosis, Transport to MC Escalator	Rat	Particles	49	Ps - First	No Effect	Inhibited by length; conc.	Stober et al. 1990 in Stober et al. (1993)
	Rat	Short Chr	14			Fibers <4 um	Yu et al. 1990
Dissolution in Extracellular Fluid	In-vitro	Chr	180	Zero	Affects rate	Diameter determines lifetime	Hume and Rimstidt 1992
	In-vitro	Crc	11,000				Zoitus et al. 1997
Transport to the Interstitium	Rat	Particles	2.3	–	–	–	Stober et al. 1990 in Stober et al. (1993)
(Component Mechanisms)							
(Phagocytosis and expulsion by epithelial cells)							
(AM phagocytosis, transport through epithelium)							

Table 6-2. Relative Rates, Half-lives for Particles Cleared by the Varous Operating Mechanisms of a Healthy Lung
(continued)

Tissue/Lung Regime	Species	Fiber Type	Particle Half-life[a] (days)	Kinetic Order	Mineralogical Effects	Size Effects	Reference
Mechanisms							
(Diffusive transport through the epithelium)							
(Forced mechanical transport through the epithelium)							
Sequestration							
(Phagocytosis and internalization by epithelial cells)							
(AM phagocytosis, immobilization due to overload)							
Pulmonary (Interstitial)							
IM Phatogyctosis, Transport to Lymphatics	Rat	Particles	2,300		Unspecified negative effect	Inhibited by length; conc.	Stober et al. (1990) in Stober et al. (1993)
	Dog	Ams	2,200				Oberdorster et al. (1988)
Diffusive Fluid Transport to Lymph			2,200				Churg 1994
Dissolution in Extracellular Fluid			(Same as Above)				
Transport to Endothelium, Pleura							
(IM phagocytosis, transport through interstitium)							
(Diffusive transport through the interstitium)							
(Forced mechanical transport through the interstitium)							
Sequestration							
(Encapsulation in granulomatous tissue)							
(Internalization by interstiial/endothelial cells)							

Table 6-2. Relative Rates, Half-lives for Particles Cleared by the Varous Operating Mechanisms of a Healthy Lung *(continued)*

Tissue/Lung Regime	Species	Fiber Type	Particle Half-life[a] (days)	Kinetic Order	Mineralogical Effects	Size Effects	Reference
Mechanisms							
The Pleura							
PM Phatogyctosis, Transport to Lymphatic Stomata							
Dissolution in extracellular fluid							
Sequestration							
(Encapsulation by granulomatous tissue)							
(Phagocytosis by mesothelial cells)							

[a]For zero order mechanisms, half-lives reported are half of the time required for complete clearance for the process that is constant with time. For first order mechanisms, the true half-lives (i.e., the time required for half of the initial population to disappear) is reported.

Particles and fibers that are deposited in the pulmonary portion of the lung may also be transported by a variety of mechanisms into and through the epithelium lining, the alveolar ducts, and alveoli to the underlying interstitium and endothelium that are located within the interalveolar septa (see Section 4.4). In those portions of the lung parenchyma that lie proximal to the pleura, such mechanisms may also facilitate transport to the mesothelium. Putative mechanisms by which such transport may occur include:

- if particles are sufficiently compact to be phagocytized by alveolar macrophages, they may be transported within macrophage "hosts" through the epithelium to the interstitium;

- if particles are sufficiently compact to be phagocytized by the epithelial cells lining the air passageways of the deep lung, they may be transported into cell interiors or transported through to the basement membrane, the interstitium, the endothelium, and (eventually) the pleura;

- particularly when associated biological effects that cause changes in the morphology of epithelial cells, particles may diffuse between the cells of the epithelium to underlying tissues; and/or

- particles may be transported through respiratory epithelium mechanically due to physical stresses associated with respiration within the lung.

Although the transport of fibers and particles from airway lumena to the interstitium is apparent in many studies (see below), the precise mechanisms by which such transport actually occurs has yet to be delineated with certainty.

Particles deposited in the interstitium can also be cleared and the processes by which these particles are ultimately cleared are similar to, but may be substantially slower than, the mechanisms by which particles deposited in airway spaces can be cleared. Such mechanisms include:

- if the deposited particles are soluble, they may dissolve and be transported away from the lungs in blood or lymph; or

- if the particles of the interstitium are sufficiently compact to be phagocytized by interstitial macrophages, they may be taken up and transported to the lymphatic system for removal.

The mechanisms by which particles that reach the pleura and mesothelium may be cleared are also similar to those operating in the interstitium:

- if the deposited particles are soluble, they may dissolve and be transported away from the lungs in blood or lymph; or

- if the particles that reach the pleura are sufficiently compact to be phagocytized by pleural macrophages, they may be taken up and transported to the lymphatic system for removal.

Particles cleared from the pleura by macrophages appear subsequently to be deposited at sites of lymphatic drainage along the pleura (i.e., at lymphatic ducts) from where they are ultimately cleared in lymph (Kane and MacDonald 1993).

The various degradation, clearance, and transport mechanisms that affect the retention of asbestos in the lung and other target tissues (identified above) exhibit disparate kinetics that may be further altered by the size, shape, mineralogy, and concentration of the particles affected. Therefore, the kinetics of these mechanisms are considered below. The mechanisms evaluated include:

- dissolution;
- muco-ciliary transport;
- macrophage phagocytosis and transport; and
- diffusional transport.

Evidence for the existence of these mechanisms and inferences concerning their kinetics derive primarily from retention studies, which may include both studies of retained structures in animals following either short-term or chronic exposure, or human pathology studies in which the lung burdens of deceased individuals are correlated with their exposure history. Other information also comes from *in vitro* studies. Various, increasingly sophisticated models have also been developed to predict the individual and combined effects of these mechanisms.

6.2.1 Animal Retention Studies

Retention studies track the time-dependence of the lung burden of asbestos or other particulate matter (i.e., the concentration of particles in the lung) during or following exposure. Thus, such studies are designed to indicate the degree to which inhaled structures are retained. Depending on the time frame evaluated, however, effects due to deposition and those due to clearance may not easily be distinguished in such studies. Moreover, due to the near impossibility of isolating the various compartments of the lung when preparing for quantitative analysis of tissue burden (e.g., the pure respiratory components vs. the larger airways or the tissues directly associated with airway lumena vs. the underlying interstitium or endothelium), it is nearly impossible to separate the effects of the various clearance mechanisms, which typically operate over vastly different time scales (Table 6-2). This is why modeling has proven so important to distinguishing effects attributable to individual mechanisms.

Results from retention studies must be evaluated carefully. In addition to the limitations highlighted above, the lung burden estimates from such studies may be affected by the manner in which asbestos is isolated from lung tissue for measurement and the manner in which the concentration of asbestos is quantified (Chapter 5). For example, lung burden estimates may vary substantially depending on what portions of lung parenchyma are sampled or whether whole lungs are homogenized. Results may also vary depending on whether lung tissue is ashed or dissolved in bleach during sample preparation. More importantly, because several clearance

mechanisms are affected by the size and even the mineralogy of the structures being cleared, studies (particularly older studies) that track lung burden by mass or by total fiber number may not adequately capture such distinctions.

6.2.1.1 Studies involving short-term exposures

The latest retention studies tend to focus on the fate of long fibers (typically those longer than 20 μm) in support of the generally emerging recognition that these are the fibers that cannot be readily cleared from the pulmonary compartment of the lung and that, not coincidentally, contribute most to disease (further addressed in Section 6.4).

Hesterberg et al. (1998a), for example, tracked the time-dependent retention in rats of two fiber categories: (1) WHO fibers[1] and (2) WHO fibers longer than 20 μm for a range of man-made vitreous fibers (MMVF's), a refractory ceramic fiber (RCF1a), and amosite following a 5-day (6 hr/day), nose-only exposure. Rats were sacrificed at intervals up to a year following exposure. The amosite was size-selected to contain a high proportion of fibers longer than 20 μm. Aerosol concentrations were also adjusted to maintain target concentrations of 150 f/cm^3 for long fibers for each sample tested. Airborne mass concentrations varied between 17 mg/m^3 for amosite to as much as 60 mg/m^3 for the other fiber types. Lungs (without trachea or main bronchi) were weighed and stored frozen. For analysis, each lung was dried to constant weight, minced, and a portion was ashed. The ashed portion was further washed with filtered, household bleach, then filtered and applied to an SEM stub. Fiber numbers and dimensions (in both aerosols and tissue) were determined by SEM with a minimum of 200 fibers counted. In addition, analysis continued until a minimum of 30 fibers longer than 20 μm were counted.

In their study, Hesterberg et al. (1998a) tracked the ratios of retained fiber concentrations with time to the concentration retained 1-day following cessation of exposure. The observed time-dependent decay in these ratios were then fit to one-pool (single first order decay) or two-pool (weighted sum of two first order decays) models. With zero time assumed to be the time immediately following cessation of exposure. The authors recognize that at least some clearance likely takes place during the 5 days of exposure so they expected the assumption that retained concentrations at the end of exposure on day 5 to be equal to deposited concentrations would cause their analysis to slightly underestimate clearance rates. They also recognized that waiting 24 hours after cessation of exposure to measure retention allows some short-term clearance of upper airways, so that they expected their analysis would better focus on slower clearance from deeper in the lung.

Results reported by Hesterberg et al. (1998a) indicate that the dimensions and concentrations of fibers in aerosols from the five synthetic fibrous materials were all similar, but that the amosite aerosol contained a substantially greater number of fibers (including the longest fibers) and that the fibers, on average, were somewhat shorter and substantially thinner than the other aerosols. Of the fibers initially deposited in the lung (based on measurements made 1 day following cessation of exposure), comparable fiber numbers of long (>20 μm) fibers were retained across

[1]WHO fibers are those longer than 5 μm, thinner than 3 μm with an aspect (length to width) ratio greater than 3 (WHO 1985).

all six fiber types. Deposited concentrations of fibers 5–20 μm in length were more variable, but values within one standard deviation still overlapped. About 6 times as many short amosite fibers (<5 μm) were initially deposited than for any of the other fiber types. The authors also indicate that the dimensions of retained fibers were generally shorter and thinner than the original aerosol and were much more similar across retained fiber types than the original aerosols.

Clearance of long fibers (>20 μm) for all six fiber types could best be described using a two-pool model. The first pool cleared relatively rapidly (within the first 90 days) and represented a minimum of 65% of the lung burden observed 1 day following exposure. The second pool cleared much more slowly. For amosite fibers in the second pool, during the approximately 275 days of clearance, retention was only reduced to 80% of the 90-day value. In contrast, all five of the synthetic fibers were reduced to less than 30% of their 90-day value during this period. For amosite, the first pool decayed with a half-life of 20 days (90%CL: 13–27) and all of the other fibers with half-lives of 5–7 days (with varying confidence bounds). For the slower pool, amosite fibers exhibited a half-life of 1,160 days (90% CL: 420–∞) with the other fibers showing half-lives varying between 24 and 179 days. The combined, weighted half-life for amosite was 418 days (90%CL: 0–1060). The authors also note that data reanalyzed from an earlier study (Hesterberg et al. 1996) indicate a corresponding weighted half-life for crocidolite of 817 days (246–∞) and indicate that this was best fit using a single exponential (a one-pool model).

Hesterberg et al. also indicate that in this and previous studies approximately 20–60% of long fibers typically clear from the lung within 2 weeks post-exposure. They further suggest that this rapid clearance may be attributable to muco-ciliary clearance from the upper respiratory tract. They further report from the present study that short amosite fibers cleared much more rapidly than long fibers. Fibers <5 μm in length were reduced by 90% in the first 90 days (in comparison to 65% for long fibers). However, from 90 to 365 days, little or no clearance was observed for amosite fibers of any length.

For four of the synthetic fibers, long fibers cleared at the same rate as short fibers (all more rapidly than amosite) and the authors report that the data suggest transverse breakage for these fibers. Moreover, they attribute the more rapid clearance of long fibers among the MMVF's to dissolution, since these fibers exhibit *in vitro* dissolution rates that are rapid relative to the time scale of macrophage clearance. One synthetic fiber MMVF34, which is a stonewool, disappeared much more rapidly than any other fiber and the long fibers disappeared more rapidly than the short fibers. MMVF34 shows the greatest *in vitro* dissolution rate at neutral pH for any of the fibers tested in ths study and dissolves particularly rapidly at pH 4.5 (the pH found in the phagosomes of macrophages). The authors postulate that clearance of all of the synthetic fibers are enhanced over amosite by dissolution and breakage.

In summary, Hesterberg et al. (1998a) observed that:

- multiple clearance mechanisms (operating over multiple time scales) contribute to clearance;
- for sufficiently soluble fibers, long fibers clear more rapidly than short fibers;

- for insoluble fibers, a subset of long fibers clears rapidly within the first few months following exposure and the remaining long fibers clear only extremely slowly, if at all;

- short fibers of all types are cleared at approximately the same rate (much more rapidly than long, insoluble fibers);

- a small, residual concentration of short fibers may not always clear and may remain in the lungs (sequestered in alveolar macrophages) for extended periods; and

- in this study, there is some suggestion that short amosite fibers clear somewhat more slowly than short fibers of the other, non-asbestos mineral types studied.

Regarding the last observation, whether this is attributable to differences in fiber thicknesses among the various mineral types, due to partial contributions (even among short structures) to dissolution, or due to a unique, toxic effect of amosite is unclear. However, the likeliest of these candidate hypotheses is that the effect is due to partial dissolution.

This general pattern of observations are consistent with the findings of most, recent retention studies following short-term exposure.

In an earlier study of similar design, Bernstein et al. (1996) evaluated the deposition and clearance of a series of 9 glass and rock wools. These authors similarly found that clearance could be modeled using a double exponential for all length fibers (in similar length categories of <5, 5–20, and >20 μm) and that for soluble fibers, long fibers clear more rapidly than short fibers (with the intermediate length fibers in between).

For the Bernstein et al. (1996) study, if one assumes that the pool of longer-lived fibers is representative of macrophage clearance, this suggests that the efficiency of clearance by macrophages decreases with increasing fiber length and that the longest structures are not phagocytized at all, so that they remain exposed to the extracellular medium where dissolution occurs. Lending further support to this interpretation, the authors also report that the clearance rate for long fibers correlate with measured *in vitro* dissolution rates at neutral pH while the clearance rates for short fibers neither correlate with *in vitro* dissolution rates at neutral pH or at pH 4.5. Although the latter pH corresponds to the pH found in the phagosomes of macrophages, there is likely too little fluid available in such organelles to support efficient dissolution. The authors also indicate that a sufficient number of fibers were counted during the study to suggest that breakage is not playing a role in clearance (except at very early times) and that the clearance rate for short fibers appears to be the same or slower than that observed for nuisance dusts.

In another, earlier study of similar design Eastes and Hadley (1995) evaluated four types of MMMF's and crocidolite. All of the samples (including crocidolite) had been size-selected to assure a large fraction of fibers longer than 5 μm. Unfortunately, due to differences in reporting, it is not possible to compare the initial loading of crocidolite fibers to those reported for amosite in the Hesterberg et al. (1998a) study. However, results from this study further support the physical interpretation of clearance suggested in the studies discussed above. In fact, the authors

report that the time-dependent size distribution of retained fibers observed in this study agree well with a computer simulation of fiber clearance. The simulation assumes that long fibers dissolve at the rate measured for such fibers *in vitro* and that short fibers of every type are removed at the same rate as short fiber crocidolite (which is practically insoluble). This is strong evidence that short fibers are cleared by macrophage phagocytosis and that long fibers cannot be cleared by macrophages, but may dissolve in extracellular fluid provided that they are sufficiently soluble.

Regarding crocidolite, the data from the Eastes and Hadley (1995) study suggest that short crocidolite fibers appear to clear at a rate that is somewhat slower than observed for any of the short MMVF fibers. Importantly, however, the interpretation of short fiber clearance in this paper is somewhat confounded because, unlike the studies discussed above, short fibers in this paper are defined as all fibers <20 μm, so there may be some confounding with MMVF fibers that are dissolving. As previously indicated, the Hesterberg et al. (1998a) work also suggests that short asbestos (amosite) fibers may clear more slowly than short fibers of differing mineralogy and Hesterberg et al. only includes fibers <5 μm in their definition. Nevertheless, it is still possible that some effects due to dissolution may still be affecting the clearance of these shorter fibers.

Surprisingly, a visual inspection of the data presented in Eastes and Hadley (1995) table suggests a lack of any long-term clearance for long fiber crocidolite (>20 μm). Yet, the authors model long fiber crocidolite clearance using a single exponential (suggesting no rapidly clearing compartment). The long-term half-life reported for crocidolite in this study is approximately 220 days (with estimated CIs of 165–566 days). This overlaps with the long-term clearance half-life reported by Hesterberg et al. (1996) for crocidolite of approximately 820 days (246–∞).

Equally surprising, Hesterberg et al. (1998a) also modeled crocidolite clearance as a single exponential, which might suggest better penetration to the deep lung by crocidolite, less clearance by muco-ciliary transport or alveolar macrophage transport, or better penetration to the interstitium than other fibers. More likely, however, it may simply indicate that the two-pool model does not represent a statistically significant improvement in model fit over the one-pool model. However, relative size distributions would need to be evaluated carefully before drawing any such conclusions. Eastes and Hadley (1995) also report clearance of short fiber crocidolite is modeled as a double exponential with short and long half-lives of 25 and 112 days, respectively. Since this fiber category contains fibers up to 20 μm in length (in this study only), this does suggest at least some contribution from muco-ciliary and alveolar macrophage mediated clearance for crocidolite.

In two studies, Coin et al. (1992, 1994) evaluated the fate of chrysotile fibers in rats exposed for 3 hours to 10 mg/m^3 (reportedly containing >5,000 fibers longer than 5 μm/cm^3). For lung analysis, the left lung was separated into peripheral and central regions under a dissecting microscope. Slices of peripheral and central portions were separately weighed and minced. Tissue was digested in sodium hypochlorite and then filtered. A quality control test indicated that the digestion process caused a slight (~10%) decrease in fiber number and slight decreases in fiber diameter and fiber length. Fiber-size distributions were evaluated by SEM. A stratified counting procedure was employed to assure equal precision for each length category of interest. Measurements for each category were then converted to mass equivalents.